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Analysis of Chlorinated Organic Compounds Formed during Chlorination of Wastewater Products

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ANALYSIS OF CHLORINATED ORGANIC COMPOUNDS FORMED DURING CHLORINATION OF WASTEWATER PRODUCTS

by

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Chemical byproducts produced during the chlorination of municipal wastewater were examined in a study that employed several specially modified analytical methodologies. Volatile byproducts were examined by the use of gas chromatography with selective detectors and gas chromatography/mass spectrometry (GC/MS). Using XAD resins for concentration of trace organics in the wastewater samples before and after chlorination, a number of chlorinated aromatic and aliphatic compounds were found after chlorination and superchlorination.

A rapid and convenient microextraction method was developed that is suitable for the analysis of trihalomethanes and other volatile halogenated organics at the microgram-per-liter level in water. Also, a computer program was developed that may be used in conjunction with a GC/MS computerized data system for the identification of polyhalogenated compounds present as minor components in a complex chemical mixture. A procedure also was developed to determine the concentrations of amino acids in wastewaters, sludges and septage, before and after chlorination. Two chlorinated derivatives of tyrosine were found in a superchlorinated septage sample. Nonvolatile compounds in natural waters and municipal wastewaters, before and after chlorination, were studied by high performance liquid chromatography. Fractions collected before chlorination of the sample showed that trihalomethane formation potential was spread throughout the natural polymer.

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FOREWORD

Nearly every phase of environmental protection depends on a capability to identify and measure specific pollutants in the environment. As part of this Laboratory's research on the occurrence, movement, transformation, impact and control of environmental contaminants, the Analytical Chemistry Branch analyzes chemical constituents of water and soil and develops and assesses new analysis techniques.

The Federal Water Pollution Control Act of 1970 requires the disinfection of all wastewater effluents. In most treatment plants in the United States, disinfection is achieved through chlorination. Recently, concern has been expressed concerning the formation of chemical by-products when chlorine is used as a disinfectant or biocide. In a five-year study, separation and identification methods were developed for volatile and nonvolatile byproducts of chlorination of natural waters and wastewaters and a number of chlorinated compounds were characterized.

David W. Duttweiler
Director
Environmental Research Laboratory
Athens, Georgia

ABSTRACT

Chemical by-products produced during the chlorination of municipal wastewater were examined in a study that employed several specially modified analytical methodologies. Volatile by-products were examined by gas chromatography with selective detectors and gas chromatography/mass spectrometry (GC/MS). Using XAD resins for concentration of trace organics in the wastewater samples before and after chlorination, a number of chlorinated aromatic and aliphatic compounds were found after chlorination and superchlorination.

A rapid and convenient microextraction method was developed that is suitable for the analysis of trihalomethanes and other volatile halogenated organics at the microgram-per-liter level in water. Also, a computer program was developed that may be used in conjunction with a GC/MS computerized data system for the identification of polyhalogenated compounds present as minor components in a complex chemical mixture. A procedure also was developed for determining the concentrations of amino acids in wastewaters, sludges and septage, before and after chlorination. Two chlorinated derivatives of tyrosine were found in a superchlorinated septage sample.

Non-volatile compounds in natural waters and municipal wastewaters, before and after chlorination, were studied by high performance liquid chromatography. Fractions collected before chlorination of the sample showed that trihalomethane formation potential was spread throughout the natural polymer. After chlorination, "total" organic halogen of a non-volatile nature was determined by adsorption of the organics on either XAD resins or powdered activated carbon (PAC) followed by elution of the resin and combustion of the eluate or by direct combustion of the PAC. In both cases, it was found that organic halogen was spread throughout the natural polymer, although chlorination at the levels used (20-30 mg/L) did not much affect the average molecular weight of the polymer.

This report was submitted in fulfillment of Grant No. R-803007 and Cooperative Agreement No. R-805822 by North Texas State University under the sponsorship of the U.S. Environmental Protection Agency. The report covers the period 1 January 1974 to 1 August 1979, and work was completed as of January 1980.

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ABBREVIATIONS AND SYMBOLS

CALCLI	--data set used to evaluate LCS program (California waste after superchlorination)
CECD	--Coulson electrolytic conductivity detector
DAI	--direct aqueous injection
EC	--electron capture detector for gas chromatography
GAC	--granular activated carbon
GC	--gas chromatography
GC/MS	--gas chromatography with mass spectroscopic detection
HALSTI	--data set used to evaluate LCS program (synthetic mixture of organic compounds)
HPLC	--high pressure liquid chromatography
LLE	--liquid-liquid extraction
LCS	--limited cluster search
S	--standard deviation (n based)
THMs	--trihalomethanes; $CHCl_3$, $CHCl_2Br$, $CHClBr_2$, $CHBr_3$
TOC	--total organic carbon
TOCl	--total organic chlorine
TOX	--total organic halogen
VCO	--volatile chlorinated organic compounds
X	--experimental value
XAD	--trade name for Rohm and Haas macroreticular resins for adsorption of organic compounds from water

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SECTION 1

INTRODUCTION

This report describes a series of research efforts whose general theme was to study the chemical by-products formed during the chlorination of municipal wastewater effluents. At the initiation of the research in 1974 little was known of this subject; since then many research groups worldwide have investigated various aspects of the question, or more broadly, the question of by-products formed during the chlorination of all types of waters. It is now well established that by-products are produced whenever chlorine is used as a disinfectant or bio-cide. Among these are the trihalomethanes, now the subject of regulations which limit their concentration in drinking water.

The research which is reported here had several objectives, some of which evolved over the project period. They were as follows:

1. to develop separation and identification methods for the determination of the types and quantities of volatile by-products produced by the chlorination of water, particularly municipal wastewater after secondary treatment. Central to this effort was the evaluation of XAD resins for concentration of trace organics in water, and the use of gas chromatography with selective detectors, and gas chromatography/mass spectrometry (GC/MS) for the elucidation of the structures of these substances. In the course of the study a rapid and convenient microextraction method was developed which is suitable for the analysis of trihalomethanes and other volatile chlorinated organics at the μg/L level in water. Also, a computer program was developed which may be used in conjunction with a GC/MS computerized data system for the identification of polyhalogenated compounds which may be present as minor components in a complex chemical mixture.

2. to develop separation methods based on high performance liquid chromatography (HPLC) for the study of wastewater effluents and natural waters before and after chlorination. The purpose of these studies was to extend our knowledge concerning the non-volatile compounds in water, and in particular, to determine whether chlorinated non-volatile halogenated products are produced by chlorination of water.

3. to investigate the chlorinated by-products produced when very large doses of chlorine (1000-3000 mg/L) are used for the treatment of wastewaters, sludges and septage. The use of such high doses has been proposed as a method for disinfection and stabilization of septage and sludge and as a possible alternative wastewater treatment scheme for small systems.

SECTION 2

CONCLUSIONS

The conclusions derived from this study are as follows:

1) Chlorination of natural waters and municipal wastewaters causes the formation of many new halogenated organic compounds.

2) These new halogenated organic compounds may be classified as purgeable-volatile; non-purgeable-volatile; and non-volatile. Based on the content of organic-bound halogen, the yields of purgeable-volatile and non-volatile organic halides are larger than those in the non-purgeable volatile category.

3) Purgeable-volatile by-products usually are dominated by the chlorine-, bromine- and iodine-containing trihalomethanes. The yield of these compounds is approximately 2-5 mole per cent based on the carbon content of the original water, providing no ammonia nitrogen is present. When ammonia nitrogen is present, yields of trihalomethanes are correspondingly lower.

4) Iodine-containing compounds are found in very small quantities relative to the bromine and chlorine containing compounds.

5) The yield of non-volatile organic halides (as measured by GAC adsorption/pyrolysis/microcoulometric procedure) is usually 2-5 times the yield of trihalomethanes.

6) The nature of the non-volatile organic compounds in water and wastewater, either the halogenated by-products or their precursors, is not known. However, size exclusion HPLC studies have shown that the molecular size of the natural organic matrix is changed only slightly upon chlorination.

7) Fractionation of the natural aquatic organic polymer by size exclusion HPLC has shown that trihalomethane formation potential is spread through the molecular size range of the polymer.

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8) Fractionation of the polymer after chlorination has shown that the non-volatile organic-bound halogen in the polymer is also spread throughout the polymer.

9) Fractionation of the natural polymer with a weak anion exchange resin with pH gradient elution produces fractions in three separate pH regions. Model compound studies suggest that one of these fractions has a pK_a value similar to that of phenols, and another the pK_a value of phenyl-carboxylic acids. The nature of the third fraction, which occurs to various extents in waters from different sources, is unknown.

10) Non-purgeable volatile organic halides are increased in yield and in number by the use of high concentrations of chlorine (2000 - 4000 mg/L).

11) The structures of the non-purgeable volatile organic halides isolated from "superchlorinated" municipal wastewater and other domestic waste products, suggests that they are derived from oxidative degradation of humic- or fulvic-acid-like precursors.

12) A special computer program has been developed for the analysis of GC/MS data. The program searches for isotope clusters in the mass spectra of GC fractions, thus assisting in the detection of new halogenated compounds.

13) Application of the new GC/MS computer program to a superchlorinated septage sample confirmed the presence of a large number of new chlorinated compounds as contrasted to the unchlorinated control sample.

14) A combined ion exchange/ligand exchange procedure has been used for the isolation of free amino acids from wastewater matrices. After isolation the amino acids are derivatized and quantified by combined gas chromatography/mass spectroscopy.

15) Analysis of a secondary treated municipal wastewater sample for amino acids has revealed only low levels of free amino acids (<5-10 µg/L).

16) Analysis of municipal sludge and septage supernatants revealed substantial amounts of free amino acids which upon chlorination are converted in some cases to chlorinated by-products.

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17) In one case, tyrosine, the amino acid is found with ring-chlorination; a mono- and di-chlorotyrosine were confirmed by GC/MS analysis.

18) Further study of the non-volatile components of natural waters and wastewaters is required to understand the mechanics of the chlorination process. In particular, spectroscopic methods, and new HPLC column modes and detectors should be applied to this study.

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SECTION 3

RECOMMENDATIONS

Further study of the non-volatile organic constituents in natural surface and ground water sources, and in treated waters and wastewaters is recommended. Particular attention should be given to the development of chromatographic and other separation processes; to the invention of new chromatographic detectors with element specificity or spectroscopic capabilities; to studies which elucidate the mechanism of the formation of chlorinated by-products during water chlorination; and to the investigation of the fate and effects of non-volatile organic compounds before and after chlorination in the aquatic environment and upon consumption by man.

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SECTION 4

BACKGROUND

Chlorine and hypochlorite solutions have been used for the disinfection of wastewaters since the middle of the nineteenth century (1). In 1884, Brewster, New York became the first American city to use chlorine as a wastewater disinfectant to protect against potential contamination of the New York City drinking water supply. By 1945, Enslow and Symans (2) had defined properly effective wastewater treatment as a three-stage process including terminal chlorination for disinfection. Active interest in terminal wastewater chlorination grew rapidly across the United States. This interest culminated in Amendments to the Federal Water Pollution Control Act (1970) (3) requiring all wastewater effluents to be disinfected. Although chlorination was not specified in the Act as the only acceptable disinfection technique, it is the one used almost exclusively in the United States.

Terminal chlorination of wastewater effluents is emphasized to achieve the following objectives (4):

1. prevention of the spread of disease,
2. protection of potable water supplies, bathing beaches, receiving waters used for boating and water contact sports, and
3. protection of shellfish and other aquatic life forms.

The minimum effective chlorine dosage necessary to achieve the above objectives was evaluated by the California State Department of Health, Bureau of Sanitary Engineering and later by the Environmental Protection Agency (5). The studies were based on fecal coliforms contained in residual effluents. The Environmental Protection Agency's temporary commitment of 200 to 400 bacteria per 100 ml of effluent depending on the nature of the receiving waters is the national guideline, although some states have set more stringent requirements (6).

CHEMISTRY OF CHLORINE

When molecular chlorine is dissolved in water, it is hydrolyzed (7) according to the equation:

$$Cl_2 + H_2O \longrightarrow HOCl + H^+ + Cl^-$$

This reaction is 99 per cent complete in only seconds, and thus the aqueous chemistry of dilute chlorine solutions is in fact the chemistry of hypochlorous acid. Indeed, chlorination reactions can be effected with equal efficiency by the use of solutions of hypochlorite salts.

Hypochlorous acid is a weak acid which dissociates according to the equation:

$$HOCl \rightleftharpoons H + OCl^-$$

The dissociation constant for HOCl is 2.95×10^{-8} at 18°C (8); thus, at a pH of 7.5 there are approximately equimolar amounts of HOCl and $OC\ell^-$ present in aqueous solution.

Kinetic studies have shown aqueous chlorination reactions to be extremely complicated. Reviews have been published by Jolley (9), Morris (10), Carlson and Caple (11), and Pierce (12). For many reactions the pH dependence of reaction rates suggests that hypochlorous acid HOCl is the reactive species or is involved directly in the generation of the principal reactive species. Hypochlorous acid is reported (12) to be 10^4 times more reactive than the hypochlorite ion $OC\ell$. However, various authors have attributed the reactive species to be the hypochloronium ion H_2OCl^+, the free chloronium ion Cl^+ (14), and the chlorine radical $Cl\cdot$ (11). Carlson and Caple (11) state that chlorine containing organic products will be derived from the attack of electrophilic species such as H_2OCl^+ or Cl^+ or by a free radical process. The former process will generate products by aromatic substitution or addition reactions, while the less-likely radical process may occur with reactants which will give a stable radical intermediate. De la Mare *et al*. (15) studied aromatic halogen substitution using low concentrations of HOCl and added perchloric acid and silver perchlorate. They concluded that the measured rate was determined by the generation of Cl^+ according to the following sequence:

$$\begin{aligned}
HOCl &\longrightarrow Cl^+ + HO^- \\
HOCl + H^+ &\longrightarrow H_2OCl^+ \\
H_2OCl^+ &\longrightarrow Cl^+ + H_2O \\
Cl^+ + ArH &\longrightarrow ArCl + H^+
\end{aligned}$$

However, in the absence of low pH and silver ion, the system became much more complicated. Hypochlorite and chloride ions which are present in solution could react to form new chlorinating species Cl_2 and Cl_2O:

$$H_2OCl^+ + Cl^- \longrightarrow Cl_2 + H_2O$$

$$H_2OCl^+ + OCl^- \longrightarrow ClOCl + H_2O$$

These products have been shown to be potent chlorinating species (16). Furthermore, at concentrations above about 0.001 M HOCl, the kinetic form showed partial dependence on the square of the HOCl concentration indicating other reactions produce even more powerful chlorinating species. Hine (14) has discussed evidence that the hypochloronium ion H_2OCl^+ is less reactive than the chloronium ion Cl^+, but may still be an important species in the chlorination of very reactive substrates such as anisole and phenol. Still, it is safe to say that at this point few definitive mechanistic studies have been reported and much uncertainty remains regarding the mechanism of aqueous chlorination processes.

At greater concentrations of chlorine, e.g. above one gram per liter (1000ppm) and at low pH, it is possible that molecular chlorine may represent an important kinetically active species. Such solutions have a characteristic yellow color which may be attributed to molecular chlorine (17).

Other important reactive species in the chlorination of natural water and wastewater are chloroamines (18); the products of reactions of ammonia (and its derivatives) with HOCl:

$$NH_3+HOCl \longrightarrow NH_2Cl+HOH$$

$$NH_2Cl+HOCl \longrightarrow NHCl_2+HOH$$

$$NHCl_2+HOCl \longrightarrow NCl_3+HOH$$

Studies of the reactive nature of chloroamines have been extensive. This work was reviewed in detail by Kovacic (18) and Jolley (19). Jolley calculated the concentrations of active chlorinating species based on equilibrium constants for the appropriate reactions. For example, a system at a pH of 7.5 containing Cl_2 and Cl^- at concentrations of 1 and 10 mg/l as Cl equivalent and containing ammonia at 1 mg/l resulted in the following concentrations of the chlorinating species:

Species	Concentration, mg/l (as Cl equivalent)
$HOCl+OCl^-$	0.0004
NH_2Cl	0.9729
$NHCl_2$	0.0264
NCl_3	trace

However, it is important to note that the specific reactivity of monochloramine is approximately 10^4 less than that of HOCl (20) thus offsetting much of its concentration advantages. Also, monochloramine is an effective aminating reagent (21), which may effectively compete with chlorination reactions. The following (22) may also be a competitive reaction:

$$NH_2Cl + NHCl_2 + HOCl \longrightarrow N_2O + 4H^+ + 4Cl^-$$

This type of reaction occurs when the free available chlorine, HOCl and OCl^-, approaches a value of approximately eight times by weight that of the available nitrogen. The mechanistic chemistry for this reaction is unclear. One should recognize that, while chloramine formation reactions produce new potential chlorinating reagents, break point chlorination results in a reduction in the total amount of chlorinating species. Break point chlorination (23) is defined as the point at which the amount of chlorine added is equal to the stoichiometric quantity required for complete conversion of ammonia to nitrogen according to the following equation:

$$3Cl_2 + 3H_2O + 2NH_3 \longrightarrow N_2 + 6HCl + 3H_2O$$

In practice break point chlorination refers to the introduction of chlorine until free available chlorine is observed by some analytical method, indicating the complete conversion of ammonia to chloroamines or other forms. Thus, break point chlorination results in a reduction of the "total available chlorine" whereas chloramine formation simply produces a shift in the ratio of "free available chlorine" versus "combined available chlorine". In systems containing ammonia and chlorine the following definitions apply to active chlorine species present:

> Free available chlorine: hypochlorous acid in its various forms including hypochlorite ion and chlorine, if present.
>
> Combined available chlorine: chloramines in all their forms which will oxidize iodide ion to iodine.

CHEMICAL REACTIONS OF CHLORINE

In discussing the chemical reactions of chlorine, the primary reactive species considered here is hypochlorous acid, HOCl. The reactions of this species have been reviewed recently by Jolley (24) who classified them into three categories:

1. Oxidation
2. Addition
3. Substitution
    - a. Formation of N-Cl compounds
    - b. Formation of C-Cl compounds
    - c. Haloform Reaction

Oxidation Reactions

Some of the most important oxidation reactions occur with other inorganic species. This class includes the following reactions:

$$HOCl + SO_3^- \longrightarrow SO_4^- + HCl$$

$$H_2O + 4HOCl + S_2O_3^- \longrightarrow 2SO_4^- + 4HCl + 2H^+$$

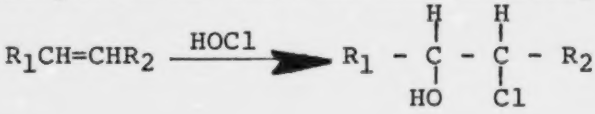
Sulfite (25) and thiosulfate (26) react instantaneously and quantitatively with all chlorinating species and thus are often used as "quenching" reagents in chlorination studies. Nitrite (27) reacts with aqueous chlorine to form nitrate according to the following equation:

$$NO_2^- + HOCl \longrightarrow Cl^- + NO_3^- + H^+$$

Other species which serve as reducing agents for hypochlorous acid include Fe^{++}, Mn^{++}, and H_2O_2 (27), and organic compounds. The chemistry of these reactions is not straightforward and has not been described in detail. These oxidation reactions probably constitute the largest category in terms of total chlorine consumption. As Jolley indicated, about 99 per cent of the reacted chlorine ends up as the reduced inorganic chloride (19).

Addition Reactions

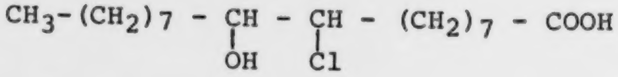
Hypochlorous acid may add to olefinic double bonds to yield chlorohydrins as shown in the following equation:



$$R_1CH{=}CHR_2 \xrightarrow{HOCl} R_1 - \underset{HO}{\overset{H}{C}} - \underset{Cl}{\overset{H}{C}} - R_2$$

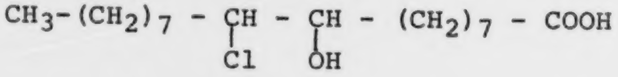
Carlson and Caple (11) have shown that aqueous chlorination of oleic acid produces a mixture of 9-chloro-10-hydroxystearic acid (III) and 10-chloro-9-hydroxystearic acid (IV).

III:



$$CH_3-(CH_2)_7-\underset{\substack{|\\OH}}{CH}-\underset{\substack{|\\Cl}}{CH}-(CH_2)_7-COOH$$

IV:



$$CH_3-(CH_2)_7-\underset{\substack{|\\Cl}}{CH}-\underset{\substack{|\\OH}}{CH}-(CH_2)_7-COOH$$

Other authors (12) have considered this type of reaction too slow to be important in dilute aqueous solution, but the data of Carlson and Caple do not confirm their expectations. However, it should be noted that few examples of addition products have been observed in "real world" surveys of chlorination products from wastewaters or municipal drinking waters.

Substitution Reactions

Reactions of HOCl with ammonia have been discussed previously. HOCl also reacts with organic amines to displace a proton and form the corresponding N-Cl bond. The reaction rate depends, in general, on the nucleophilicity of the nitrogenous substrate (20). Reactions of HOCl with amides (13) usually require more vigorous conditions than those available under normal wastewater chlorination. Most N-Cl bonds are relatively unstable in aqueous media. For example, dichloroamine and trichloroamines are reported to decompose to nitrogen and hypochlorous acid (23):

$$2NHCl_2 + H_2O \longrightarrow N_2 + HOCl + 3H^+ + 3Cl^-$$

and dichloroamino acids decompose to nitriles and/or aldehydes depending on the ratio of amino acid to chlorine, the pH, and other factors (28, 29, 30):

$$R-\underset{\substack{|\\NCl_2}}{CH}-COOH \longrightarrow RCN + RCHO$$

The second, and most important, group of substitution reactions are characterized by displacement of a proton in a carbon-hydrogen bond to form a carbon-chlorine bond. These reactions require activation of the leaving proton before the reaction will proceed under typical wastewater chlorination conditions. Substrates such as activated aromatic systems or alpha, alpha'-diketomethylene groups are required for successful reaction. Soper (31) was one of the earliest to study such a mechanism, describing phenolic substitution by chlorine in 1926. Aromatic substituents such as hydroxy, alkoxy, and amino which are strongly electron donating activate the ring to chlorination, while electron withdrawing groups such as nitro, carbonyl, cyano, and positively charged ions retard the chlorination process (32).

Haloform Reaction

An especially important group of substitution reactions known as haloform reactions have been known since 1822 (33). These are complex reactions which ultimately yield trihalomethanes such as chloroform. Morris (10), Morris and Baum (34), and Pierce (12) have reviewed the basic chemistry of haloform formation. Several types of substrates are known, most commonly those which contain the methyl keto group $CH_3C = O$, and those which can be easily oxidized to methyl keto forms; such as secondary alcohols.

Morris and Baum (34) have discussed the classical reaction pathway for the haloform reaction shown in the following scheme (Figure 1). This scheme includes several observations known about the reaction, such as the fact that it is base catalyzed, and that bromide and iodide may react with hypochlorus acid to be incorporated into halogenated products. Morris and Baum give six functional atom group arrangements (Figure 2) they feel would give haloform products, each of which easily forms the carbanion intermediate. They speculate that many of these groupings are in humic substances found in water. Major chloroform yields were obtained for twelve model compounds with the six functional groups, including chlorophyll which contains the pyrrole group. Hoehn (35) *et al.* also suggested that the addition of algae or more *specifically* the chlorophyll-a accounts for the additional THM's observed during summer months. It should be noted that rapid electrophilic substitution produces the initial intermediate I which opens under the influence of base to form the Carbanion II. Morris and Baum (34) have pointed out that stable carbanion formation is a prerequisite for haloform production, and that precursors such as m-dihydroxy aromatic compounds are more reactive than simple methyl ketones for this reason.

The presence of chloroform generated by chlorination of a wastewater was first reported by Glaze and co-workers (36). The first extensive treatment of the presence of haloforms was conducted by Rook (37). It should be noted that, following Rook's lead, the term "haloform reaction" is applied to any series of aqueous chlorination reactions which produces haloforms, in this case, $CHCl_3$, $CHCl_2Br$, $CHClBr_2$ and $CHBr_3$. This not only includes the traditional haloform reaction characterized by the chlorination of alpha-keto methyl groups but also the polychlorination of aromatic systems, followed by ring rupture to result in haloform production. Rook (38) initially recognized the correlation of the bleaching effect of water

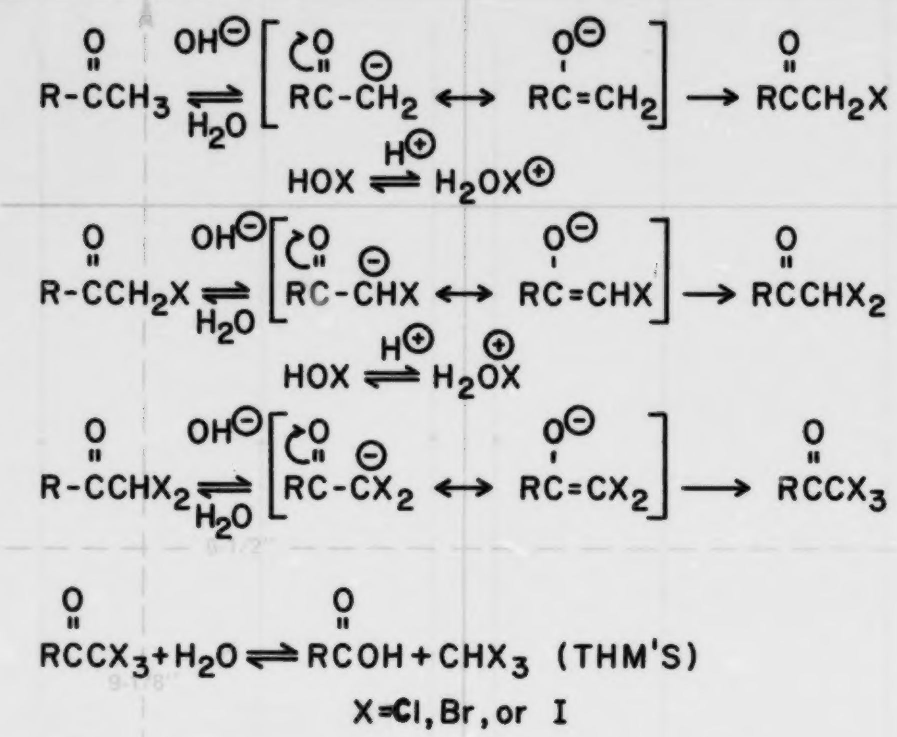

R-CCH3
OH⊖
H2O
RC-CH2
RC=CH2
RCCH2X
H⊕
HOX ⇌ H2OX⊕
R-CCH2X
RC-CHX
RC=CHX
RCCHX2
R-CCHX2
RC-CX2
RC=CX2
RCCX3
RCCX3+H2O ⇌ RCOH + CHX3 (THM'S)
X=Cl, Br, or I

Figure 1. Haloform mechanism for methyl carbonyl compounds after Morris and Baum (34).

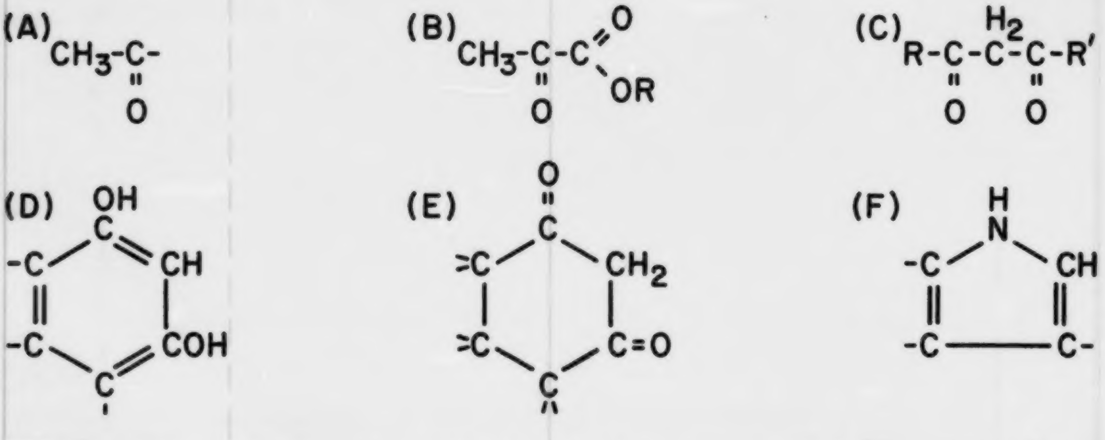


Figure 2. Structural units identified by Morris and Baum (34) as THM precursors.

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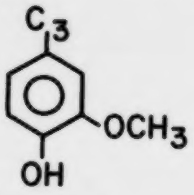
chlorination with the appearance of haloforms. He noted that the coloration of the water was caused by

> "humic substances which are very stable to biological decay and do not appreciably diminish in concentration during impoundment (of the water supply). These substances are the products of plant decay and include macromolecules which are condensation products of quinones and polyhydroxybenzenes, with substituent NH_2 groups".

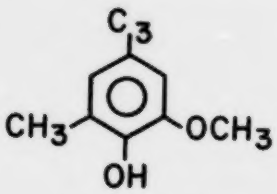
His laboratory experiments demonstrated that the chlorination of purified humic substances dissolved in doubly distilled water produced haloforms. Recently, Glaze et al. (39) and Schnoor et al. (40) have shown that fractionated fulvic acids from natural waters yield trihalomethanes. In view of the importance of aquatic humic substances in this regard, the following sections discuss in more detail the properties of these substances as they relate to the formation of haloforms and other halogenated organic by-products.

SOIL AND AQUATIC HUMIC SUBSTANCES

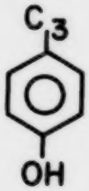
Much of the earth's carbon is found in the form of woody tissue, a major component of which is lignin. Lignin is a mixed polymer which appears to have only three structural units, guaiacyl, syringyl, and p-hydroxyphenylpropane.



GUAIACYL

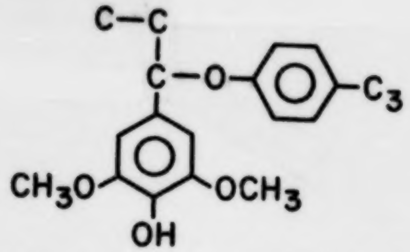


SYRINGYL



p-HYDROXYPHENYLPROPANE

Apparently, these units are incorporated into the lignin polymer by carbon-carbon or carbon-oxygen-carbon linkages with β-4'-ether linkages predominating (41).



EXAMPLE OF β-4$'$-ETHER LINKAGE BETWEEN SYRINGYL AND p-HYDROXYPHENYLPROPANE UNITS.

It has also been proposed that most C_3 side chains are methyl ketone, allyl, and secondary alcohol configurations (42).

Although lignin is a relative refractory material towards biodegradation, certain microorganisms, particularly fungi, are capable of degrading the lignin polymer. This process, which is not fully understood, is a part of the so-called humification process which results in the deposition of organic substances called humus in soil and water. Among the various hypotheses regarding the synthesis of humic substances, Martin and Haider (43) prefer the following. Lignin molecules are degraded to smaller phenolic units which together with simple phenolic substances synthesized by microorganisms, plant and microbial proteins, carbohydrates, and other substances in the soil, are combined by autoxidative and enzymatic polymerization to form humus. A portion of this humus is sufficiently water soluble so that it is eventually leached into ground and surface waters and provides the bulk of the carbon content of these waters.

Historically, humic substances have been separated into four substances by the scheme shown in Figure 3. Table 1 gives the properties of the three soluble fractions as determined by several different methods. It is clear that the terms "humic acid", "fulvic acid", and "hymatomelanic acid" do not refer to monodisperse substances. Rather these are strictly operational terms referring to the products obtained by the scheme shown in Figure 3. The amount and type of each fraction will depend on several parameters such as the amount and type of vegetation contiguous to the origin of the humic substance, ambient factors such as temperature, soil or water type, the presence of soil or aquatic microorganisms, etc. (42).

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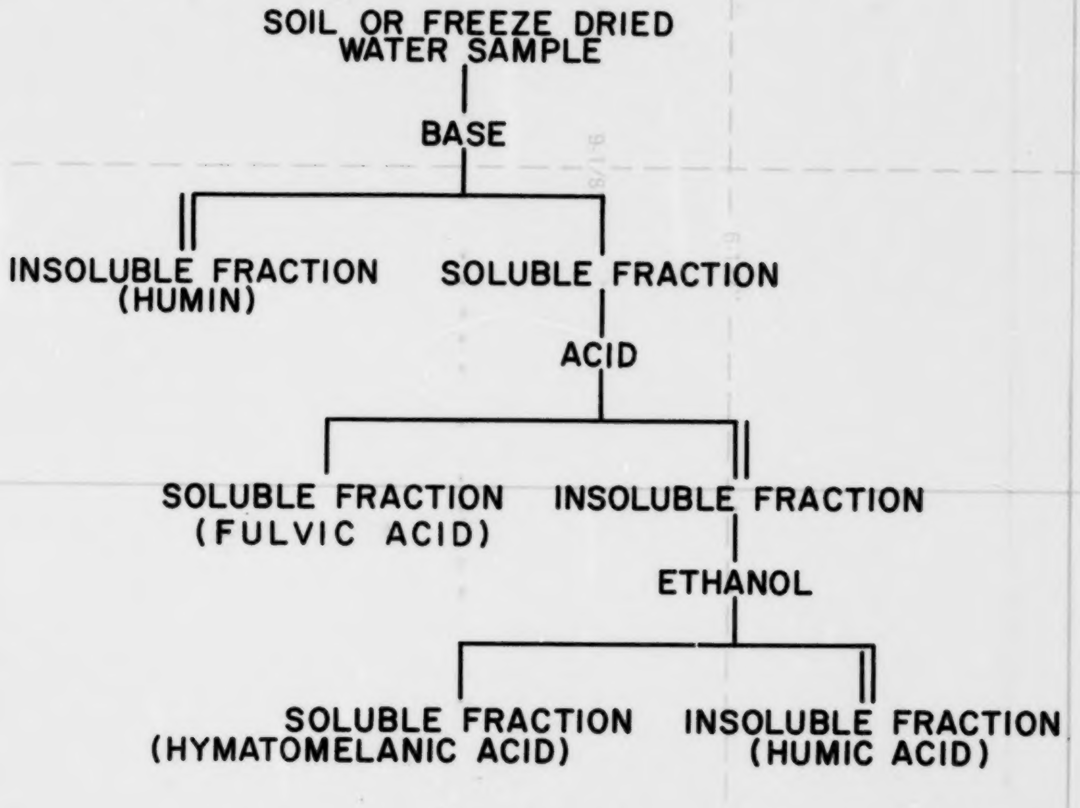
SOIL OR FREEZE DRIED WATER SAMPLE
BASE
INSOLUBLE FRACTION (HUMIN)
SOLUBLE FRACTION
ACID
SOLUBLE FRACTION (FULVIC ACID)
INSOLUBLE FRACTION
ETHANOL
SOLUBLE FRACTION (HYMATOMELANIC ACID)
INSOLUBLE FRACTION (HUMIC ACID)

Figure 3. Separation scheme for soil and aquatic humic substances (42).

TABLE 1. PROPERTIES OF COMPONENTS OF HUMIC SUBSTANCES

Group Name	Solubility (44)	M.W. Range of Average
Fulvic Acid	Sol. in NaOH & Mineral Acid	200 - 1,000 (44) 200 - 300 (45) a. 951 (41) a. 688 (41) a. 668 (41) c.
Hymatomelanic Acid	Sol. in NaOH & Alcohol; insol. in Mineral Acid	
Humic Acid	Sol. in NaOH insol. in Mineral Acid and Alcohol	up to 200,000 (44) 700 - 26,000 (45) c. 1,300 - 13,000 (45) 30,000 - 80,000 (45) d. ∿1,000 (41) e. 1,684 (41) b. 4,500 - 26,000 (41) c. 14,000 - 200,000 (41) f. ∿53,000 (41) g. ∿36,000 (41) h. 47,000 - 53,000 (41) a.

a. Osmometry
b. Freezing Point Depression
c. Diffusion
d. Ultracentifugation & Light Scattering
e. Isothermal Ditillation
f. Gel Filtration
g. Sedimentation
h. Viscosity

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The precise chemical composition of humic and fulvic acids* is still largely unknown. Schnitzer and co-workers (42) have contributed most to present knowledge in this area, but there remains much doubt regarding not only the nature of the building blocks which make up these natural polymers, but also the secondary and tertiary nature of the polymers themselves (46). The following is not meant to be a thorough review of the available information on this subject; rather it is a summary of knowledge pertinent to the discussion of water treatment practices which are effected by the presence of "humic substances" in water.

Structure of Aquatic Humic Material

A general measure of the amount of aquatic humic substances is dissolved organic carbon (DOC) or total organic carbon (TOC). Some typical TOC values in (mg/L) are shown in Table 2 (44), for four types of water: ground, sea, surface, and wastewaters.

TABLE 2. TOTAL ORGANIC CARBON RANGES FOR SEVERAL WATER TYPES (44).

Water Type	TOC (mg. per liter)
Ground	0 - 2
Sea	0.5 - 5
Surface (NORS)	3.5 (average) 1 - 20 (range) 300 (maximum)
Waste	10 - 20 (average) 1000 (maximum)

Aquatic organic matter is largely of the "fulvic" type, i.e. it is soluble in both acid and base (47), ranging from 58 - 90 per cent in seventeen samples studied by Christman and co-workers. The elemental composition of aquatic fulvic acid (FA) is shown in Table 3, taken from Alexander and Christman (41). Schnitzer and Khan (42) propose that aquatic FA contains more oxygen and less nitrogen than typical soil humus, but as Table 3 shows, there is considerable variation in FA composition depending on the source water.

---

*Hymatomelanic acid is often combined with humic acid in this discussion.

TABLE 3. ELEMENTAL ANALYSES OF AQUATIC HUMIC FRACTIONS (41)

Fraction	% C	% H	% N	% O	Ref.
Fulvic Acid	55.61	5.91	2.13	a	27
Fulvic Acid	54.87	5.56	2.41	a	27
Fulvic Acid	59.32	6.75	1.22	a	27
Fulvic Acid	58.42	6.18	1.26	a	27
Fulvic Acid	57.91	6.11	1.34	a	27
Fulvic Acid	57.08	6.47	2.17	a	27
Fulvic Acid	58.39	5.81	0.57	a	27
Fulvic Acid	41.50	5.72	1.98	50.80	18
Fulvic Acid	46.2	5.9	2.6	45.3	29

a: By Difference

Characterization of the chemical content of aquatic fulvic acids has been carried out by two general methods; chemical degradation and spectroscopic studies. Christman (41) and Schnitzer and Khan (42) have reviewed both methods. Separation of aquatic fulvics has been carried beyond the scheme shown in Figure 3, using various chromatographic techniques. Until recently, it was generally agreed that FA and HA are largely aromatic in carbon type. This conclusion was based on products obtained by oxidative and reductive degradations (42). Recent spectroscopic data (48) suggest, however, that the aliphatic content of FA may be much higher than expected, due possibly to the loss of aliphatic units during the workup of degradation products.

Schnitzer and co-workers (42) have preferred a hydrogen bonded structure shown in Figure 4 as the principal structural type in fulvic acid. Christman and Ghassemi (49) prefer the structure shown in Figure 5. The latter includes more non-carboxylic units and more aliphatic components of an unspecified type, and is a covalently bonded macromolecule. Wershaw and co-workers (50) have discussed a structure for FA in terms of a hierarchy of moieties, in the lowest level of which are the simple phenol, quinoid, and other small molecular units. These are grouped together by covalent bonds into small polymers with molecular weights of a few thousand or less. Groups of small polymers can then be linked together into aggregates by intermolecular forces such as hydrogen bonds. The degree of aggregation is a function of water pH, the oxidation state of the molecules, etc. Wershaw's model may be as precise as one can be regarding the generalized structure of aquatic humic matter. To be more specific, one must specify the precise origin of the FA, its pH, and other ambient factors. Whether a more specific generalized structure of FA can be written as preferred by Christman and Ghassemi (49), must await further research.

Reaction of Chlorine with Aquatic Humic Substances

Several research groups continue to investigate the reaction of chlorine with carbonaceous substances in water. Rook (51, 52) has suggested that the active sites within FA molecules are 1,3-dihydroxybenzenes, and the mechanism shown in Figure 6 is suggested to account for the formation of THMs and other halogenated compounds.

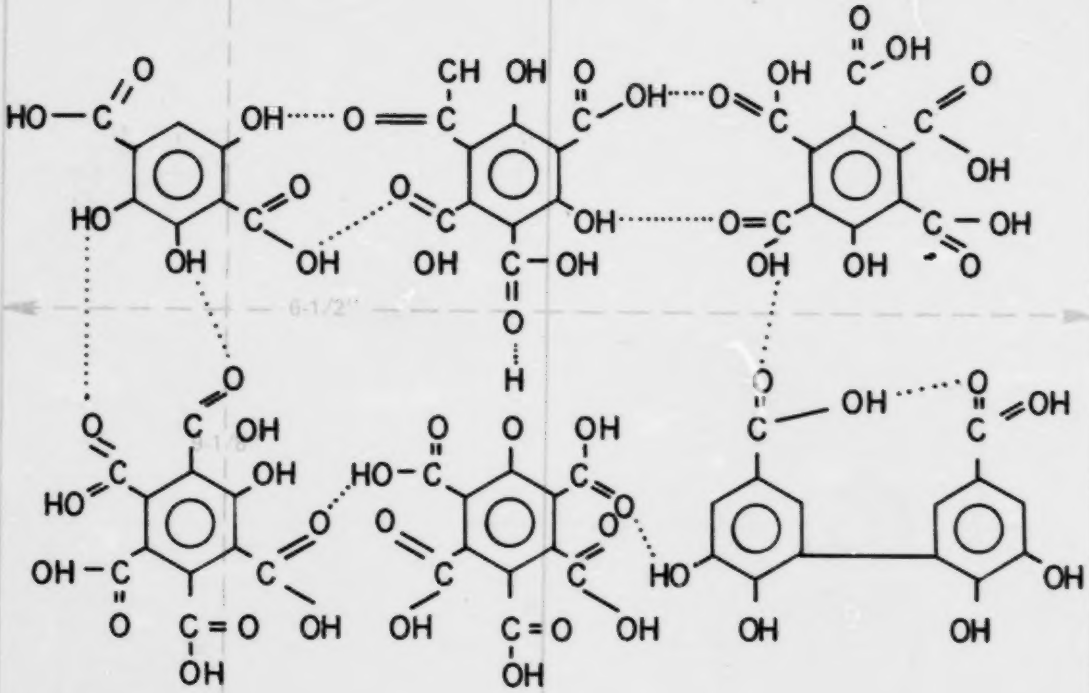

Figure 4. Structure of fulvic acid as proposed by Schnitzer (42)

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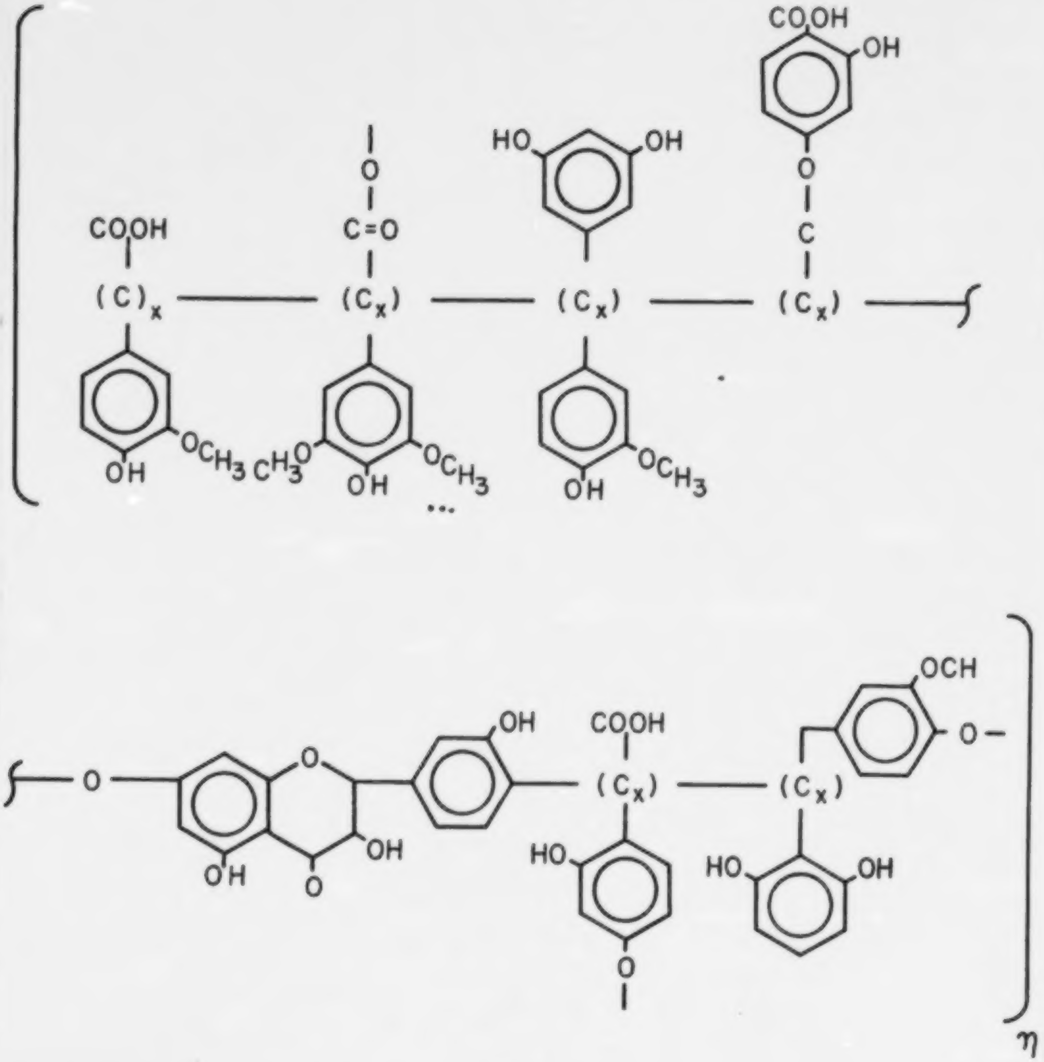
COOH
OH
HO
OH
O
C=O
COOH
O
C
(C)x
(Cx)
(Cx)
(Cx)
OH
OCH3
CH3O
OH
OCH3
OH
OCH3
OCH
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COOH
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(Cx)
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OH
OH
O
HO
HO
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η

Figure 5. Structure of humic acid as proposed by Christman and Ghassemi (49).

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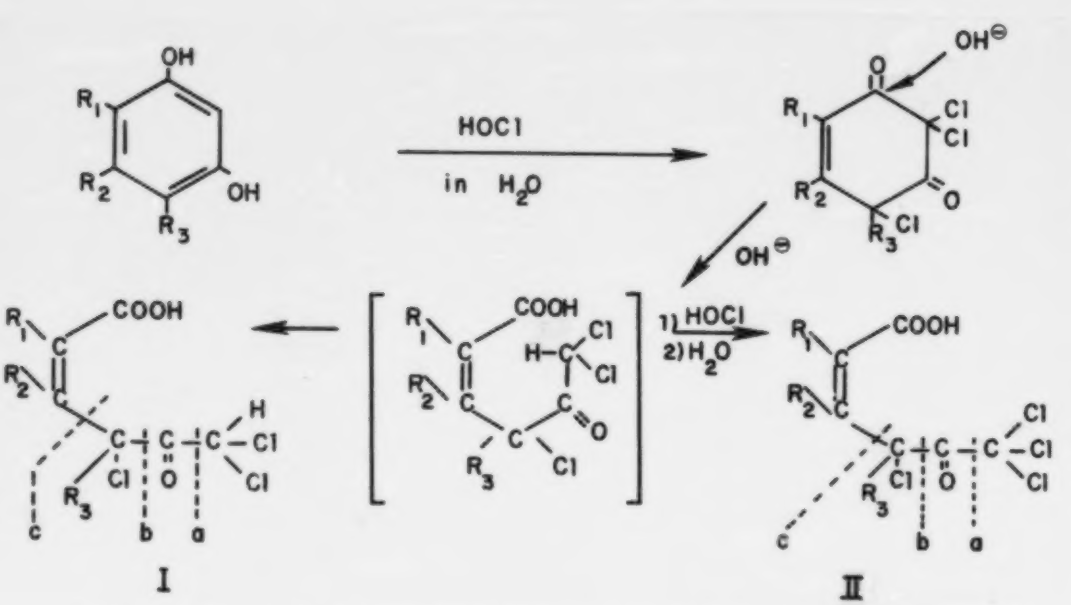
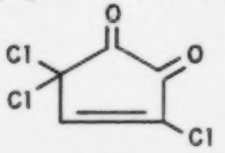


Figure 6. Rook-Moye mechanism for the aqueous chlorination of resorcinol (51).

Structures I and II yield upon cleavage, dichloromethane (Ia), dichloroacetic acid (Ib), trichloroacetone (Ic), chloroform (IIa), trichloroacetic acid (IIb) and tetrachloroacetone (IIc) respectively. Further chlorination of fragments was proposed to account for polychlorinated acetones (52).

Christman and co-workers have isolated 3,5,5-trichlorocyclopent-3-ene-1,2-dione (III) and a number of other chlorinated species from the chlorination of resorcinol (53) and aquatic humic material (54). They point out that (III) is not consistent



III

with Rook-Moye mechanisms. In fact, it should be noted there is no clear evidence that 1, 3-dihydroxybenzene moieties are the principal precursors of THMs. As shown by Morris and Baum (34), the haloform reaction is possible with any one of a number of substrates such as the β-diketones and pyrolles. Morris and Baum have shown that acetogenins (natural pigments) such as phloroacetophenone are potent haloform precursors. More recently, Arguello *et al*. (55) list a total of thirty-four substances which give low to high yields of chloroform upon aqueous chlorination.

SEPARATION AND IDENTIFICATION OF SPECIFIC ORGANIC COMPOUNDS IN WASTEWATERS BEFORE AND AFTER CHLORINATION.

The tremendous number of organic species with widely divergent structures combine to produce an incredibly complex organic matrix in wastewater effluents. Feng (56) indicated that the study of the chlorination of sewage would perhaps be impossible because of the complexities of such systems. Geiger (57) recently emphasized the point:

> "Such investigations are hindered by two intrinsic properties of organic water constituents. First, the organic assemblages in environmental samples are of an extra-ordinarily high compositional complexity; and second, single components occur in trace quantities only".

Organic content of wastewaters was historically evaluated in terms of gross parameters such as volatile solids, suspended solids, dissolved solids, Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD). Unfortunately, these parameters do not provide information on the specific chemical compounds present in the wastewater. To obtain this type of information, it is generally necessary first to separate the components of the sample into major fractions. Separation methods commonly used include differentiation by solubility (liquid-liquid extraction), adsorption (with activated carbon or resins), membrane permeability (reverse osmosis and ultrafiltration), and volatility (purge techniques). For volatile compounds further separation may be achieved by gas chromatography; for non-volatile compounds the corresponding technique is high performance liquid chromatography (HPLC).

Identification of specific organic components of the isolated fractions may be accomplished by GC or HPLC by correlation of the retention time of the unknown with that of a known compound. A higher level of assurance may be obtained by the use of selective GC or HPLC detectors such as the electron capture GC detector. For ultimate proof of structure, these techniques should be combined with spectroscopic data on the compounds in the GC or HPLC eluates. Most commonly, this is mass spectroscopic data, although infrared absorption spectra may also be useful in many cases.

Several works have been published which utilize combinations of gas chromatography and mass spectrometry (GC/MS) for the determination of specific chemical constituents of natural waters and wastewaters. References (58) and (59) include many examples.

In 1973 and 1975, Glaze *et al*. (36, 60) used these techniques for the determination of by-products produced by the chlorination and superchlorination of municipal wastewaters. About the same time, Jolley (61) developed an analytical procedure using high pressure liquid chromatography and ^{36}Cl-labeling to identify polar compounds containing carbon-bound chlorine (9, 61) formed by water chlorination. Tables 4 and 5 list the small halogenated molecules identified by these two groups.

Several chlorination studies have been done whereby compounds known to occur in municipal wastewaters (secondary effluents) were subjected to chlorination in a distilled water matrix under various treatment conditions. For example, Carlson et al. (32) demonstrated facile chlorine uptake by such compounds as phenol, anisole and acentanilide. In the same report, it was also demonstrated that biphenyl formed various chlorinated analogues upon aqueous chlorination. Increased chlorine doses resulted in formation of increasing polychlorinated analogues. Also found were chlorohydrins formed upon chlorination of some olefinic systems such as oleic acids.

Four conferences have been held to discuss the impact of water chlorination and the proceedings of the first three conferences are published (62-64). The contents of these publications include several other reports on the by-products formed during water chlorination. In addition, several papers on this subject are included in references (58) and (59).

TOXICITY OF CHLORINATED WASTEWATER EFFLUENTS.

It is beyond the scope of this report to discuss the potential toxicities of municipal wastewater effluents before and after chlorination. It is clear, however, that chlorination of water causes the formation of some new compounds which have toxic properties. The danger of this situation as pointed out by Bunch (65) is that drinking water supplies originating from surface water sources may be composed of some fraction of reconditioned sewage. To compound this problem, some areas of the world face the prospect of declining water supplies and will be forced to consider direct reuse of treated wastewater.

In addition to the potential effects of chlorinated by-products on humans, there is the concern that chlorination by-products may be detrimental to aquatic life in streams receiving wastewaters (62-64).

Several reports have appeared on the toxicity and mutagenicity of chlorinated compounds and extracts from chlorinated waters and wastewaters (62-64, 66). These studies generally take one of two paths. In one, the wastewater is tested directly, or after only gross fractionation. No attempt is made to determine the active chemical species, only to ascertain if the test water or fraction is toxic, mutagenic, teratogenic, etc.

TABLE 4. CHLORINATED PRODUCTS IDENTIFIED IN CHLORINATED WATER BY JOLLEY (61)

5-Chlorouracil
8-Chlorocaffeine
8-Chloroxanthine
5-Chlorosalicylic acid
2-Chlorophenol
4-Chlorobenzoic acid
3-Chlorobenzoic acid
4-Chlororesorcinol
4-Chloro-3-methylphenol
5-Chlorouridine
6-Chloroguanidine
2-Chlorobenzoic acid
4-Chloromandelic acid
4-Chlorophenylacetic acid
4-Chlorophenol
3-Chlorophenol
3-Chloro-4-hydroxybenzoic acid

TABLE 5. CHLORINATED PRODUCTS PRODUCED BY WATER CHLORINATION, BY GLAZE _ET AL._, (36,60)

Chloroform
Dichlorobutane
Chlorocyclohexane
o-dichlorobenzene
p-dichlorobenzene
Pentachloroacetone
Dichloroethylbenzene
N-methyl-trichloroaniline
Trichlorophenol
Dichloromethoxytoluene
Trichloroethylbenzene
Dichloro-bis(ethoxy)benzene
Trichloro-α-methyl benzyl alcohol
Trichlorocumene
Dichloroaniline derivative
Dichloroacetate derivative
Tetrachloropthalate derivative
Dibromochloromethane
3-chloro-2-methylbut-1-ene
Chloroalkyl acetate
Tetrachloroacetone
Chloroethylbenzene
Hexachloroacetone
Chlorocumene
Dichlorotoluene
Chloro-α-methyl benzyl alcohol
Trichloromethylstyrene
Dichloro-α-methyl benzyl alcohol
Trichloro-N-methylanisole
Tetrachlorophenol
Tetrachloroethylstyrene
Dichloroaromatic derivative
Trichlorophthalate derivative

The second approach seeks to identify the individual species in the effluents, then applies various toxicological tests on these compounds to evaluate the hazard associated with their presence. Garrison _et al_. (67), who used this approach, pointed out:

> "Knowledge of the specific compounds discharged is needed to study health effects of pollutants, to help determine the sources of compounds found in drinking water surveys and to establish effluent guidelines".

Geiger (57) also used this approach stating:

> "Since most biochemical reactions show a very pronounced structural dependence, studies on chemical ecology necessitate analyses for single constituents".

SURROGATE METHODS VERSUS SPECIFIC COMPOUND IDENTIFICATION.

While it is desirable to determine the precise composition of molecular types in a wastewater effluent, at least two factors make this goal unachievable.

(1) Raw and treated waters may contain thousands of compounds at trace levels;

(2) Many of these compounds cannot be identified and quantified by available analytical methods such as GC/MS.

For these reasons, water quality is often measured by surrogate or group parameters such as total (or dissolved) organic carbon TOC (or DOC); total organic nitrogen (TON), chemical oxygen demand (COD), etc. In view of the toxic nature of many organohalogen compounds (68), a TOX parameter would seem to be of value in assessing the quality of raw and treated waters.

Three approaches have been proposed for TOX measurement (69-72), each of which involves a preconcentration step and the subsequent determination of organic halogen in the preconcentrate. Since organic halogen may not be determined nondestructively, the sample preconcentrate is usually pyrolyzed to convert the halogen to halide ion in aqueous solution. The halide may then be titrated by potentiometric or coulometric methods or determined with a halogen sensitive electrode (73). Pyrolysis of organohalogen compounds to form hydrogen halides may be done reductively or oxidatively; in either case the product is almost entirely hydrogen halide (71) if the pyrolysis temperature is above 800°C.

Preconcentration of the water sample has been carried out by adsorption of the chlorinated organics on activated carbon (69, 70, 73) or XAD resins (71, 74, 75) or by extraction into a non-polar organic solvent (72). Each method has its limitations due to inefficient adsorption or interferences from inorganic halogen or other species.

Several recent investigations have shown by the use of these methods that non-volatile compounds which cannot be determined by GC or GC/MS are formed in abundance by the chlorination process. Kühn, Sontheimer and co-workers (69, 70) have shown that non-volatile activated carbon adsorbable organohalogen compounds exceed volatile compounds (in halogen content) by a factor of two to four in typical chlorinated surface water supplies in Europe. Glaze et al. (75) and Oliver (74) used XAD-resin adsorbents to obtain similar results. The relative yields of THMs and non-volatile TOX has been shown to be a function of pH and dose of halogen (70). Indirect evidence for the occurrence of non-THM products, such as those proposed by Rook (51) comes from the results of direct aqueous injection THM analysis discussed in Section 8. The larger yield of THMs found by this procedure, as compared to other methods, has been taken as evidence for decomposition of chlorinated non-volatile THM precursors in the GC injection port (76, 77). Thus, while THMs are the major volatile products which arise by the chlorination process, under certain conditions they represent a minor yield of organic halogen when non-gas-chromatographable material is taken into account.

SECTION 5

COMPUTER ASSISTED GC/MS ANALYSIS OF ORGANIC COMPOUNDS IN MUNICIPAL WASTEWATER PRODUCTS BEFORE AND AFTER CHLORINATION

INTRODUCTION

This study of municipal wastewater products before and after application of chlorine was conducted in order to determine the extent to which chlorination causes the formation of volatile organohalogen compounds. Because of the harmful potential of many chlorinated organic compounds reviewed in the previous section, any source which discharges these compounds into the environment poses a possible threat to man and other forms of life.

Gas chromatography (GC) is the most powerful separation method available for determination of volatile organic compounds. Combined with element specific detectors, GC is capable of detecting extremely small quantities of organic substances. For example, with the electrolytic conductivity detector one can detect as little as 50 picograms (10^{-12} g) of a chlorinated organic compound such as carbon tetrachloride. For the separation and identification of unknown volatile organics in complex mixtures, the most advanced method available is GC combined with on-line mass spectrometric detection (GC/MS). Both GC/MS and GC with element-specific detection have been used in this work.

The advancement of GC/MS technology was aided immensely in 1968 with development of the first fully computerized GC/MS system by Hites and Biemann (78). Their work showed examples of how an on-line computer can acquire and process GC/MS data from complex samples which have more than one-hundred GC peaks. Today specialized computer programs have been developed to simplify the data processing and/or to extract grossly obscured, relevant information from the data.

One such computer program has received various names in the literature: "Limited Mass Search (LMS)", "Extracted Ion Current Profile", or "Mass Chromatography". This is a data manipulation technique applied to GC/MS data subsequent to its acquisition and storage. The technique is used to identify the locations of specific compounds or classes of compounds within a total ionization chromatogram (TIC). The computer program extracts the ion current intensities from each spectrum in the TIC at a specific mass which is characteristic of a compound or class of compounds. These intensities are then plotted as relative ion current intensity versus spectrum number. The LMS can then be compared to the TIC to determine which peak(s) is (are) due to a particular compound or class of compounds. An example is shown in Figure 7. The top chromatogram is a TIC of a septage extract. The bottom chromatogram is the corresponding LMS at m/e 149. Almost all phthalate esters have a characteristic base peak at m/e of 149. After LMS, the phthalate esters are conveniently marked, and each specific ester can be identified by its complete fragmentation pattern. Thus, comparison of the chromatograms facilitates the location and identification of the phthalate esters.

This technique has become common during the past several years. Recent literature cites examples of its application to determine phthalate esters (79), polynuclear aromatic hydrocarbons (80), mononuclear aryl hydrocarbons (81), and many other types of compounds. Another important application of this technique, recently reported, was to determine the location of chlorinated organics in water extracts (82). The m/e of 35 was used, a mass which is highly specific for chlorine. It is unlikely that any other elemental combination with an m/e of 35 would form, but unfortunately not all chlorinated compounds produce this fragment (i.e., some chloroaromatics). Figure 8 shows an example: the TIC for a "superchlorinated" septage extract with the corresponding LMS at m/e of 35. The peaks shown in the LMS chromatogram show a high correlation with the presence of chlorinated organics in the TIC.

This section describes a new GC/MS/DS computer program which manipulates acquired and stored data to produce a "Limited Cluster Search" (LCS). The resulting LCS chromatogram indicates the peaks in a TIC which possess a specific number of chlorines and/or bromine atoms. The program is similar to Limited Mass Search programs in that it extracts specific information from each and every mass spectrum in a TIC. This information can then be plotted on a relative basis versus spectrum number for comparison with the TIC. Peaks appearing in the LCS chromatogram have a high probability of containing a given number of chlorine and/or bromine atoms.

As Aston (83) recognized in 1920, chlorine contains a mixture of isotopes with masses, respectively, of 35 and 37 and occurring naturally in a ratio of approximately 3 to 1 (^{35}Cl to ^{37}Cl). This is reflected in the mass spectra of chlorinated compounds. An ion possessing a chlorine atom will

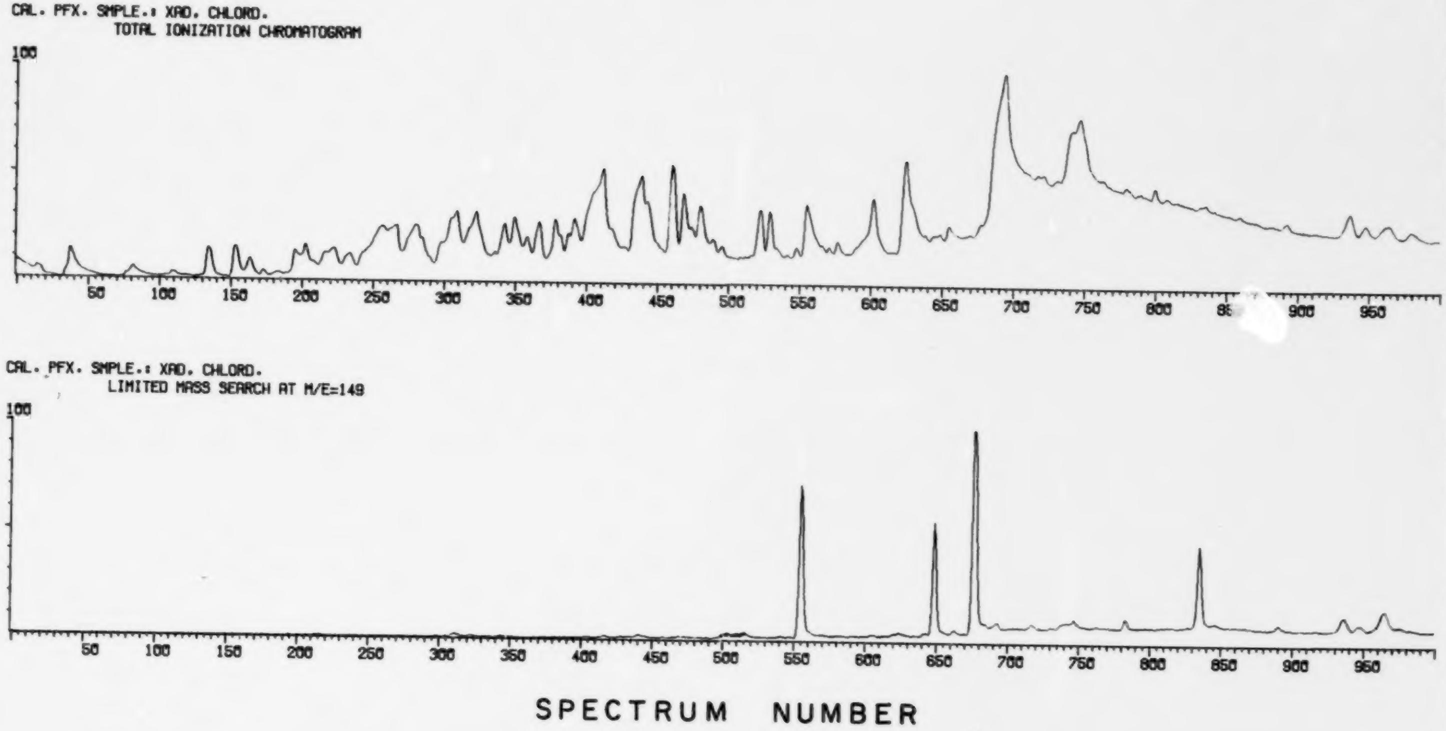


Figure 7. Total ion (top) and LMS chromatograms at m/e 149 (bottom) for XAD extracts of superchlorinated septage.

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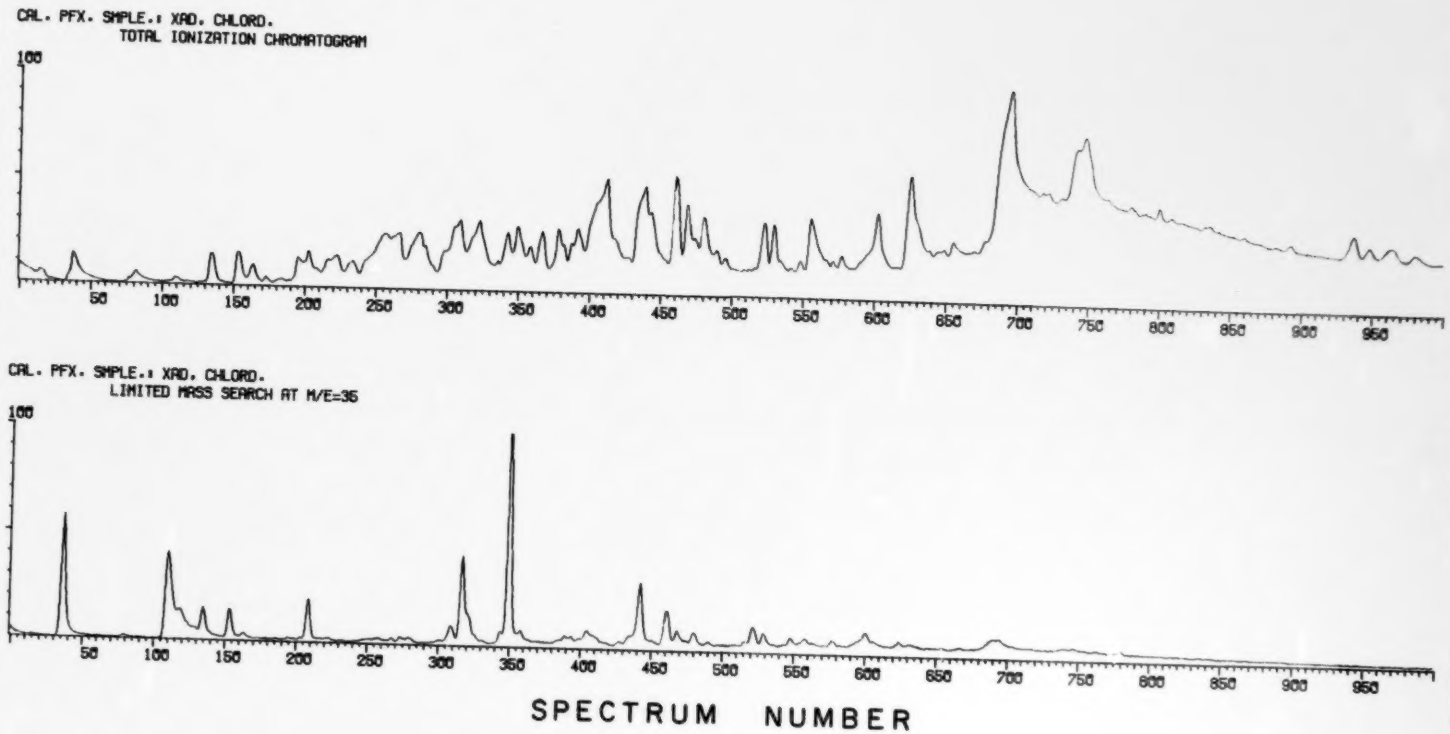

Figure 8. Total ion (top) and LMS chromatograms at m/e 35 (bottom) for XAD extracts of superchlorinated septage.

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produce two peaks at masses X and X + 2 corresponding to the respective masses of the ions with the ^{35}Cl and ^{37}Cl isotopes. The ratio of intensities of the X to X + 2 peaks will be approximately 3 to 1. An ion possessing more than one chlorine atom will produce a "cluster" of peaks. The number of peaks in the cluster will be n + 1, where n is the number of chlorine atoms. The intensities of each peak in the cluster can be calculated by expanding the simple binomial expression, $(a + b)^n$ where a and b are the relative abundances of the light and heavy isotopes. Tables have been published (84) which show the relative ion intensities of the peaks in clusters for varying numbers of chlorine atoms.

Bromine is also a mixture of two isotopes, with masses of 79 and 81, respectively. The naturally occurring ratio for these isotopes is approximately 1:1. Tables for the clusters of fragments containing multiple bromine atoms are calculated in an analogous manner to chlorine clusters and have been published (84). The relative intensities of ion fragments containing mixtures of bromine and chlorine atoms also can be calculated, and the resulting tables have been published (84).

The "Limited Cluster Search" program searches a predetermined range of masses for the appropriate isotopic cluster. An important characteristic of this program is that it is not mass specific, that is, the recognition of a chlorinated or brominated compound does not require that the isotope cluster occur at specific masses in the mass spectrum. This means that a compound can possess a wide variety of additional functional moieties and still be recognized as containing chlorine and/or bromine.

Two papers have been published which describe computer programs that can identify chlorinated and/or brominated isotopic clusters regardless of mass. Mun, et al. (85) have extended the McLafferty "Probability Based Matching" program for this purpose. The goal is to identify the number of chlorines and/or bromines in the compound, and the computer program in its present state is designed to be applied to individual spectra of relatively high quality. For GC/MS data, the appropriate background spectrum should be subtracted from the spectrum of interest before it is subjected to computer analysis. And, since there is no quantitative measure of the ion clusters, the program could not be easily adopted to produce a chromatographic profile showing relative peak intensities.

Regnier and Canada (86) have published the description of a computer program which does produce a chromatographic profile of the chlorinated and/or brominated compounds in a GC/MS data set. However, their goal was to use the relative heights of corresponding peaks in the different profile as a new identification technique. The relationship between these peak heights tends to be specific for a particular compound. It should be noted that the goal of the LCS computer program described in this chapter is to improve the ability of the analyst to find mass spectra of chlorinated and/or brominated compounds produced from the GC/MS analysis of matrices. Regnier and Canada have never reported the application of their computer program to such a sample for such a purpose. In fact, the examples they use in their report would not adequately test the ability of their program to process data in a complicated matrix which contains many interfering nonchlorinated analytes. The most complex samples which they analyzed were standard polychlorinated biphenyl mixtures. Although these samples produce a complex chromatogram, the individual peaks are closely related homologues of each other. This means that the fragmentation process for the different analogues will be very similar, and the spectra of overlapping GC peaks will tend to reinforce the isotopic clusters as opposed to interfering with them.

EXPERIMENTAL

It is well known that GC/MS, even with the most sophisticated data analysis, usually cannot be applied directly to the analysis of dilute water solutions. The technique generally is not sensitive enough to directly detect compounds of interest which are present in the parts-per-billion (μg/L) concentration range or below. Moreover, many compounds such as certain phenols, carboxylic acids and amines must be derivatized to neutral forms before high resolution GC is possible. Thus, various preconcentration, derivatization, and separation processes are utilized on environmental samples before GC/MS analysis. To separate relatively non-polar compounds from water, one commonly uses liquid-liquid extraction (87), purge-and-trap (88), or adsorption techniques (89). This section emphasizes the use of adsorption with synthetic macroreticular polymers as the preconcentration method for volatile compounds. Liquid-liquid extraction and purge-and-trap techniques for highly volatile ("purgeable") compounds are compared in a later section.

Chlorination of Denton, Texas, Municipal Wastewater

Sample Collection--

Most of the water samples used in this study were collected at the Denton Municipal Wastewater Treatment Plant. This plant utilizes a biologically activated sludge treatment process as shown in Figure 9. The treatment process includes the following:

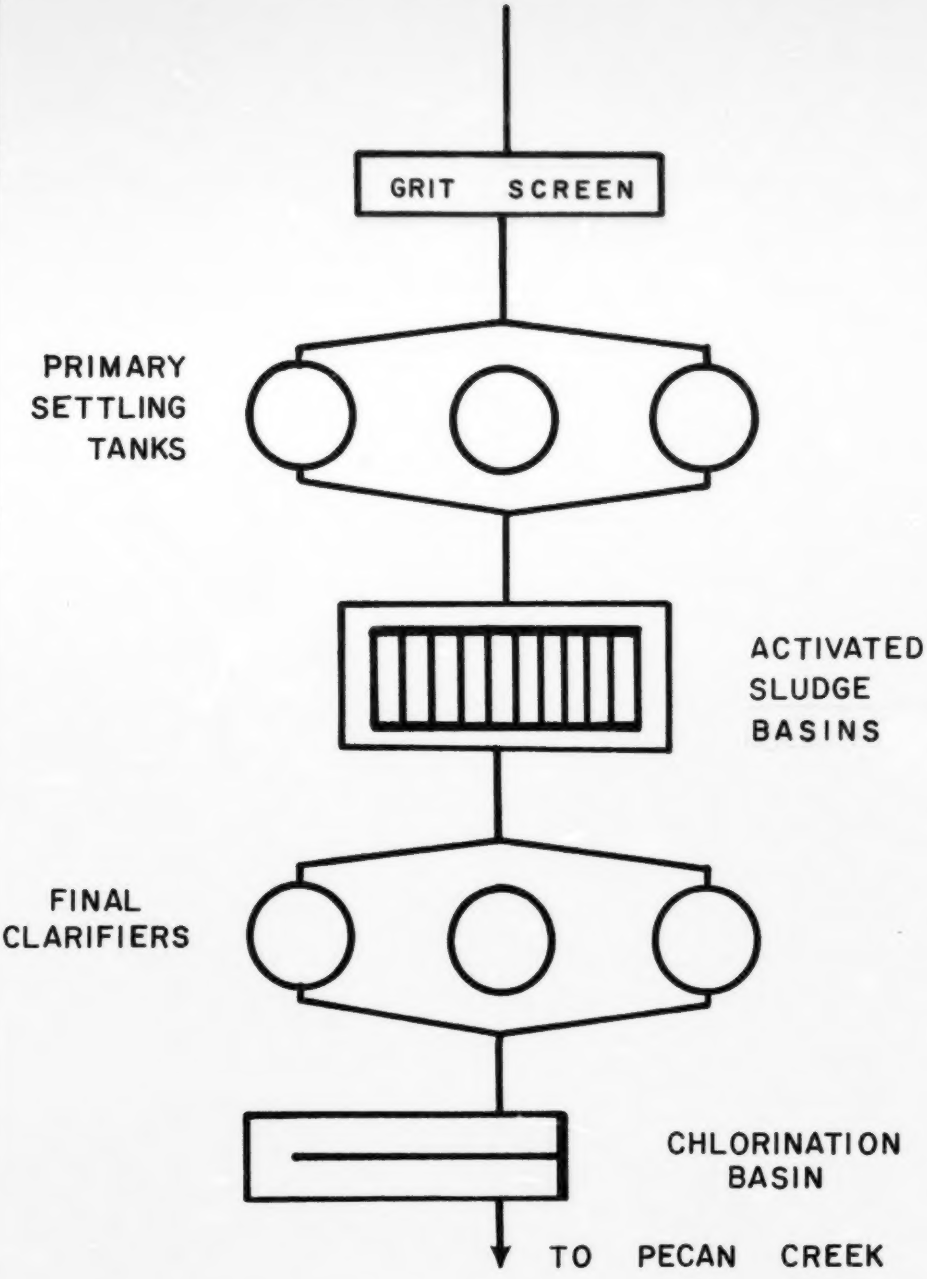
GRIT SCREEN
PRIMARY SETTLING TANKS
ACTIVATED SLUDGE BASINS
FINAL CLARIFIERS
CHLORINATION BASIN
TO PECAN CREEK

Figure 9. Schematic of the Denton, Texas municipal wastewater treatment plant.

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1. Primary clarification;
2. Digestion by activated sludge;
3. Secondary clarification;
4. Terminal disinfection by chlorination.

During most of the sampling, the plant was operating near capacity, at approximately 5.7 million gallons/day. BOD levels of the final plant effluent before chlorination averaged approximately 19 mg/L during most of the sampling period, although daily records were not kept.

In the earliest studies, samples were collected before and after the chlorination process. The specific sampling point for the chlorinated sample was at a spillway at the effluent end of the chlorination basin. The unchlorinated control sample was collected at the effluent point of one of the secondary clarifiers.

Later, however, only the unchlorinated sample was collected and transported in a steel container to the laboratory where the actual chlorination was conducted. This alternative procedure allowed the chlorinated and control sample background matrices to be identical prior to beginning the experimental procedure. The alternative procedure also allowed quality control procedures to be performed on all reagents used in the experiments.

The general approach used to demonstrate the formation of new organochlorine compounds was to analyze concentrated extracts of the chlorinated wastewater by gas chromatography using a halogen-specific detector, the Coulson electrolytic conductivity detector. This profile chromatogram could then be compared to a chromatogram of the unchlorinated control sample extract. The additional peaks seen in the chlorinated extract chromatogram represented the formation of new halogen-containing species.

These experiments published in 1973 in the Journal of Chromatographic Science (36) clearly demonstrated that new chlorinated organic compounds were generated using wastewater disinfection procedures. The experiments were followed with another series of experiments to identify the new chlorinated species. The same sampling procedure was used, but the chlorine doses were in the 1,000 to 4,000 mg/L range. These large doses were used to increase the concentrations of the chlorinated species, making their identification easier.

A second reason for conducting these studies at such high chlorine doses was to simulate a treatment process that is described in a 1969 patent (90). This process uses pressurized chlorine at similar concentrations as a means of oxidizing and disinfecting wastewater and sludge by-products. The purpose of the process is to increase the settling ability of the suspended materials, as well as to disinfect the entire substrate. The process operates by pumping the substrate into a chamber that is pressurized with chlorine. A portion of the supernatant is then pumped off into a second chamber where oxidation by chlorine is completed. A portion of the reactor mixture, approximately 75 to 80 per cent, is recycled into the first chamber where it is subjected to further oxidative chlorination. The process can be used in place of anaerobic digestors for sludge treatment, or it can even be used on the entire plant influent as an alternative to conventional treatment systems (i.e., activated sludge, trickling filter).

Chlorination and XAD-Extraction of Effluent Samples--

When the wastewater samples arrived in the laboratory, they were processed according to the scheme shown in Figure 10. The water was first filtered using coarse, fluted filter paper (Whatman #41, Whatman Limited), followed by suction filtration using Gelman #61694 glass fiber filters. Two 10-liter aliquots were then transferred into 20-liter glass vats that were equipped with glass stirring mechanisms.

Chlorination of one of the aliquots was effected by bubbling chlorine gas (Dixie Chemical Company, 99.5 per cent purity) into the sample as it was stirred vigorously. The chlorine concentration was monitored by removing aliquots, making proper dilutions, and assaying for chlorine by using the orthotolidine arsenite method (91). The chlorine contact time was one hour, after which time excess granular sodium sulfite was added to both chlorinated and control water portions. The pH of both was then adjusted to 2-to-3 with concentrated H_2SO_4.

The organic compounds in the sample were concentrated using Amberlite XAD-2 resin (20-50 mesh, Rohm and Haas Company). The resin is a polystyrene/divinyl benzene copolymer that is manufactured in the form of spherical beads (92). The porous nature of the beads results in a fairly high surface area of 300 m^2/gm (89). The surface of the resin is extremely hydrophobic, which accounts for its affinity for non-polar organics in waters. Upon contact with organic solvents, the surface is said to "relax", readily releasing most adsorbed organics. This system has a major advantage over conventional liquid-liquid extraction methods in that a much higher water-to-extractant ratio can be achieved, resulting in higher concentration factors and therefore higher sensitivity. The system has a similar advantage over carbon adsorbants. The higher adsorption efficiency of the XAD resin for most non-polar organics means that a relatively smaller amount of adsorbant can be used as compared to the amount of carbon required. This means that less

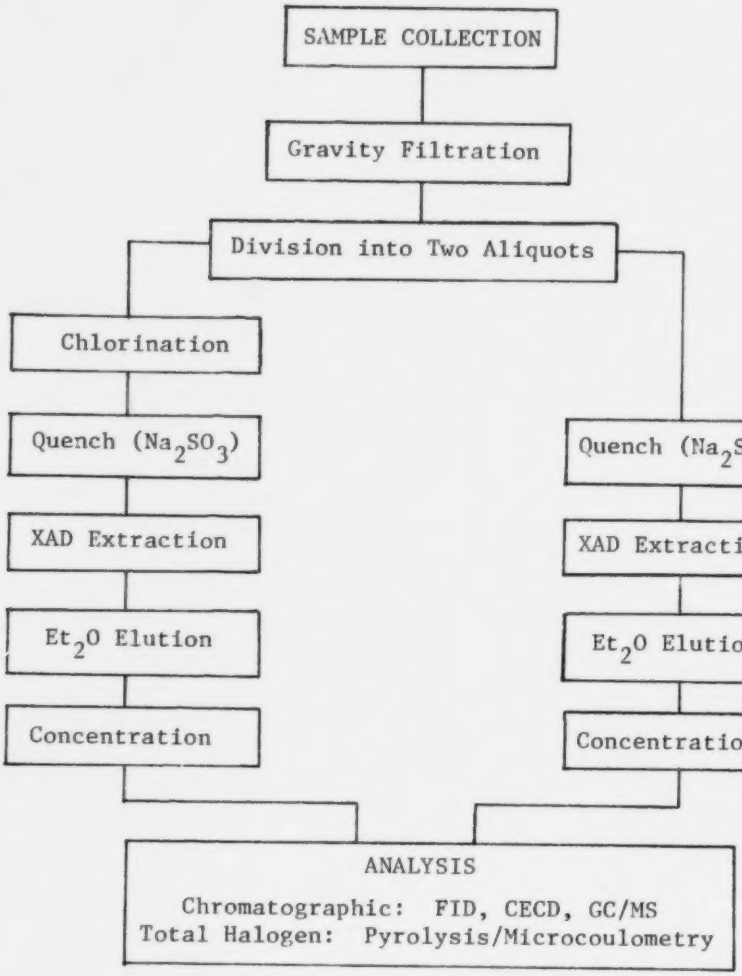
SAMPLE COLLECTION
Gravity Filtration
Division into Two Aliquots
Chlorination
Quench (Na_2SO_3)
XAD Extraction
Et_2O Elution
Concentration
Quench (Na_2SO_3)
XAD Extraction
Et_2O Elution
Concentration
ANALYSIS
Chromatographic: FID, CECD, GC/MS
Total Halogen: Pyrolysis/Microcoulometry

Figure 10. Scheme for the extraction of organic compounds from wastewaters.

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organic solvent can be used to remove the organics from the adsorbant, resulting in higher water-to-solvent ratios and thus higher sensitivity.

Careful attention was paid to the cleaning and preparation of the resin prior to its use as an analytical adsorbant. The cleaning techniques have been described in detail previously (111). Approximately 25 gm of resin was transferred from its shipping container to an erlenmeyer flask. The resin was then covered with approximately one inch of reagent-grade methanol (Fisher, ACS Certified). The erlenmeyer was then swirled several times, which dissolved unreacted monomer and suspended polymeric fines that were generated during manufacturing and shipment. The excess methanol was then removed by suction using a glass tube attached to an aspirator. Another portion of methanol was then added to the erlenmeyer, and the process was repeated until the methanol remained clear upon vigorous swirling.

Following the washing, the resin was transferred to a soxhlet extractor and extracted sequentially with methanol (Baker, b.p. = 65°C.), acetone (Baker, b.p. = 56°C.), and diethyl ether (Mallinckrodt,b.p. = 34°C.) for 24 hours with each solvent. According to Junk, *et al*. (89), the decreasing boiling points are important in avoiding cracking the resin beads with sudden, large changes in temperature.

After the extraction process had been completed, the resin was washed into a clean erlenmeyer flask using methanol, and it was stored under a portion of methanol in the stoppered flask.

The apparatus used to contain the resin during the extraction process is shown in Figure 11. After cleaning the apparatus with chromic acid, a small plug of glass wool was placed in the bottom of the column, as shown in the Figure. Approximately 1 gm of the resin, in a methanol slurry, was then transferred into the column, and the methanol was removed by allowing approximately 0.5 L of distilled water to flow through the column. Next, the column was slowly and carefully backflushed with Milli-Q water by stoppering the top of the column, placing the effluent end of the column in a beaker of distilled water and attaching an aspirator to the takeoff arm on the column. The backflush flowrate could be controlled by manipulating the stopcock on the takeoff arm. This procedure suspended the resin beads in the water in the column. The beads could then be packed in an extremely regular matrix with virtually no "channeling" effects, by quickly switching the aspirator from the takeoff arm to the effluent end of the column, removing the stopper at the top of the column, and momentarily opening the effluent stopcock. The column was then ready for use in the extraction process.

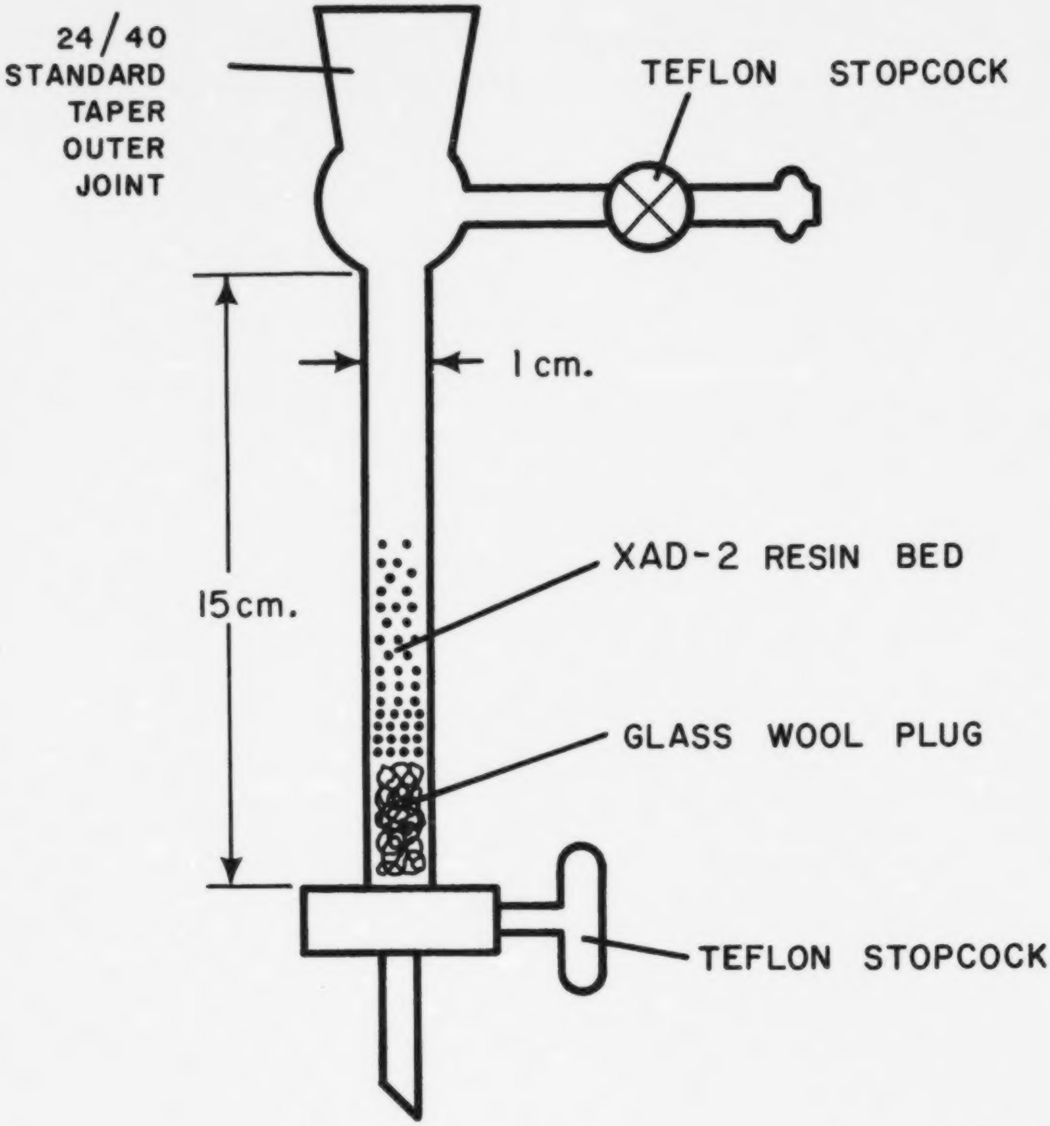
24/40
STANDARD
TAPER
OUTER
JOINT
TEFLON STOPCOCK
1 cm.
15 cm.
XAD-2 RESIN BED
GLASS WOOL PLUG
TEFLON STOPCOCK

Figure 11. Glass apparatus containing XAD-2 resin for extraction of organic compounds from water.

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The resin columns were attached to a glass siphon that descended from the glass vats. The water samples were allowed to flow through these columns at a rate of approximately 30 ml/min. The excess water was then forced out of the column using a pipette bulb. The resin was eluted with 25 ml of diethyl ether (Mallinckrodt, prepurified), which was glass-distilled in the laboratory prior to use. The diethyl ether extracts were concentrated to 1 ml using a modified Kuderna-Danish flask and a Snyder three-ball distillation column, as described by Junk (89).

The survey chromatographic analyses were then performed on the concentrates, followed by further concentration to approximately 100-50 μl for gas chromatographic-mass spectrometric analysis.

Survey Gas Chromatographic Analysis--

The survey chromatographic analyses were performed on a Varian 1800 gas chromatograph that is equipped with a flame ionization detector (FID) and a Coulson electrolytic conductivity detector (CECD). The glass chromatographic column was 6 feet by 2-mm i.d., packed with 3 per cent Dexsil 300 GC, coated on 100/120 mesh Supelcoport. The helium carrier gas flow rate was 30 ml/min. The temperature-program conditions were, as follows:

1. Isothermal at room temperature (approximately 27°C.) for four minutes after injection;
2. Program ballistically from room temperature to 50°C;
3. Program from 50° to 300° at 6°/minute;
4. Isothermal at 300°C. until no further peaks eluted.

The injector temperature was 225°, and the detector temperature was 300°C. The Coulson block temperature was 300°C. and the furnace temperature was 830°C. The detector was operated in the reductive mode with 80 mL/min. of hydrogen added to the gas chromatographic column effluent before pyrolysis. The bridge current was 30v; the attenuation was X4 to X32 (as indicated in the Figures). The FID detector was run at a range of 10^{-11} amp/mv, and an attenuation of X8 to X32 (as indicated in the Figures).

GC/MS Analysis--

The GC/MS system was a Finnigan Model 3200, controlled by a Finnigan Model 6100 computerized data system. The chromatographic conditions were the same as those described for the survey analyses. Successive mass spectral scans were acquired from m/e = 35 to m/e = 450 at a scan rate of approximately 1 sec/decade.

Analysis of Superchlorinated Septage and Sludge Supernatants

In the second portion of this study, samples of raw and superchlorinated septage pumpings were obtained from Ventura, California. The septage had been treated at the plant site by a Purifax reactor for disinfection and stabilization of the septage. Samples were shipped to the North Texas State University (NTSU) laboratory through arrangements by officials at USEPA/MERL (Cincinnati).

Sample Treatment--

Raw and superchlorinated samples of septage pumpings, the latter treated in Ventura, California, with excess Na_2SO_3 to quench were centrifuged with a Sharples centrifuge to obtain a clear centrifugate. Samples were analyzed by a modified Bellar purge and trap procedure (88) coupled to the GC/MS system. Quantification of the purge and trap data is not reported, however, since the samples were not shipped in headspace-free containers, nor were precautions taken to preserve purgeable compounds at the NTSU laboratory. Control (raw) and superchlorinated samples were also extracted by the XAD-2 resin procedures, as described previously. Ether eluates were analyzed by GC/FID, GC/CECD, and GC/MS. GC/FID data are not reported and GC/CECD data were used only to indicate halogen-containing species in the analysis of GC/MS data. Samples of XAD-2/Et_2O eluates were also combusted in the Dohrmann MT-20 pyrolysis furnace, and the furnace effluent titrated by microcoulometry. Total organic halogen values so obtained were compared with the sum of GC/MS-identified peaks.

Limited Cluster Search Mass Spectroscopy

Test Samples--

Two samples were run for demonstration of the new computer program. The first sample, HALSTI, was an artificial mixture of halogenated compounds and nonhalogenated normal alkanes. The names and sources of the compounds are listed in Table 6. The sample was prepared by adding approximately 250 microliters of each compound to 2 milliliters of acetone. 0.5 microliter aliquots of this mixture were then injected into the GC/MS for analysis. This sample was used to study the decision parameters in the computer program.

TABLE 6. NAMES AND SOURCES OF COMPOUNDS IN HALSTI SAMPLE

Name	Source, Grade
Bromoform	Fisher, Certified
n-Decane	Fisher, Certified
1,3-Dichlorobenzene	Mallinckrodt, 98% or
1,2,4-Trichlorobenzene	J.T. Baker, Practical
1,3-Dibromobenzene	Fisher, Practical
Hexachlorobutadiene	MCB, Practical
n-Tetradecane	Fisher, Reagent

The second sample, CALCLI, was the XAD-2 extract of superchlorinated California septage as described earlier in this section. The ether eluant was concentrated to approximately 100 microliters, and a 2.5 microliter aliquot of that concentrate was injected into the GC/MS system. Only a portion of the TIC (scans 290 through 510) was processed by the computer program. The first spectrum of these data was selected so that enough data would be processed to accurately reflect the ability of the program to extract important information. The entire RGC was not processed because of the computer CPU time limitations. The evaluation of each spectrum required about 0.75 minutes of computer time. Thus, for the limited data selected, the complete computer run took almost 2-1/2 hours.

The GC/MS data were transferred to Finnigan magnetic tape cassettes for long-term storage and transportation to the computer system described below for the actual computer processing.

LCS Computer Program--

Details of the Limited Cluster Search computer program are given in Appendices A and B.

RESULTS AND DISCUSSION

Denton, Texas Municipal Wastewater Extracts

Figure 12 shows typical FID survey chromatograms of the chlorinated and control wastewater extracts (chlorine dose, 2,000 mg/L). The chromatograms are typical of what one would expect from such a complicated matrix as wastewater. Well over 100 peaks are identifiable, many of which are mixtures of two or more components. Figure 13 shows typical Coulson (CECD) survey chromatograms. More than thirty halogenated species detected in the chlorinated portion are not present in the control, or they are present at distinctly lower concentrations. Some of these chlorinated compounds are the same as those generated at chlorine concentrations at 10 mg/L (36). The GC/MS total ion chromatogram (Figure 14) is similar to the FIC chromatogram. Inspection of the mass spectra reveals some thirty or more chlorinated compounds not present in the control or present in distinctly lower concentrations. Table 7 lists the compounds that are identified as species generated by chlorination.

Most of the compounds identified thus far are aromatic derivatives. The compounds are by no means derivatives of "activated" aromatics in every case, however. The chloro derivatives of benzene, toluene, ethyl benzene and benzyl alcohol do not necessarily suggest a mechanism of direct chlorination, as we shall note later. Moreover, of particular interest is the formation of several nonaromatic derivatives, such as chlorocyclohexane, a chloroalkyl acetate, and, perhaps most significant, three chlorinated acetone derivatives. The latter may be precursors of chloroform, which was shown in a previous work (60) to be formed in wastewater chlorinations, and which has been shown by other workers to result from the chlorination of organics in drinking waters (51).

Finally, the concentrations of the compounds listed in Table 7 are in the microgram-per-liter range; however, it should be noted that the sum of the concentrations shown in the right hand column of Table 7 (786 µg/L) does not represent the total concentration of chlorinated organics. Rather, this sum is a lower limit in view of the inefficiency of the various steps in the analytical procedure, and, in particular, because the procedure does not detect non-volatile species.

Ventura, California Septage Extracts

Figure 15 is the reconstructed total ion chromatogram for the extract from a sample of superchlorinated Ventura, California septage. Organics in the sample were extracted by XAD-2 resin previously and eluted with diethyl ether, as described previously. Peaks in the chromatograms that show evidence of halogen clusters are shaded. Peaks that give a response on the Coulson electrolytic conductivity detector are indicated by a "C"; and those that have an ion at m/e 35 are indicated by the number 35 (cf. Figure 8). Figure 15 also designates peaks found in the control (unchlorinated) sample

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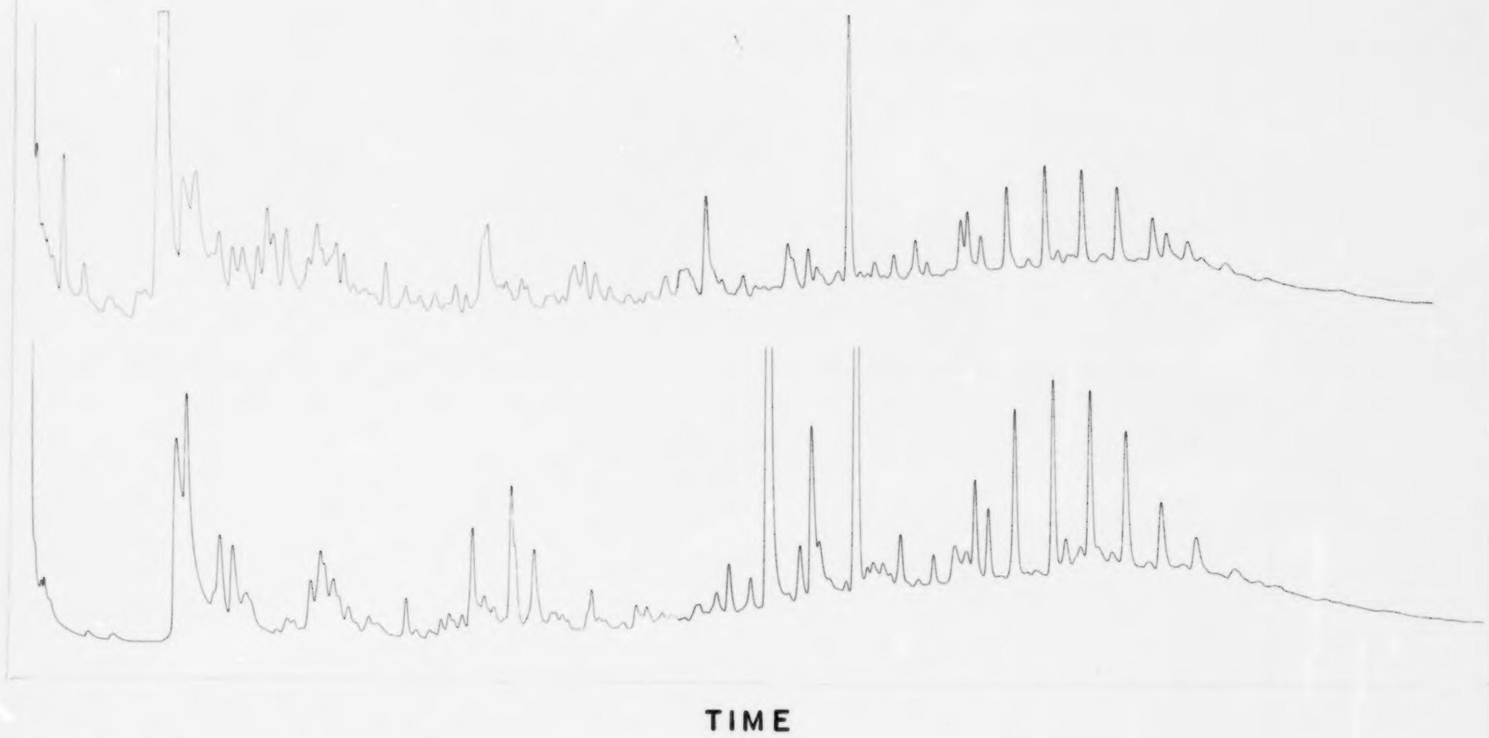

Figure 12. Gas chromatograms (FID, 16×10^{-11} amp/fs) of Denton, Texas wastewater extract. Bottom, before chlorination,; top, after 2,000 mg/L chlorination for 1-hour contact period. Analytical conditions described in text.

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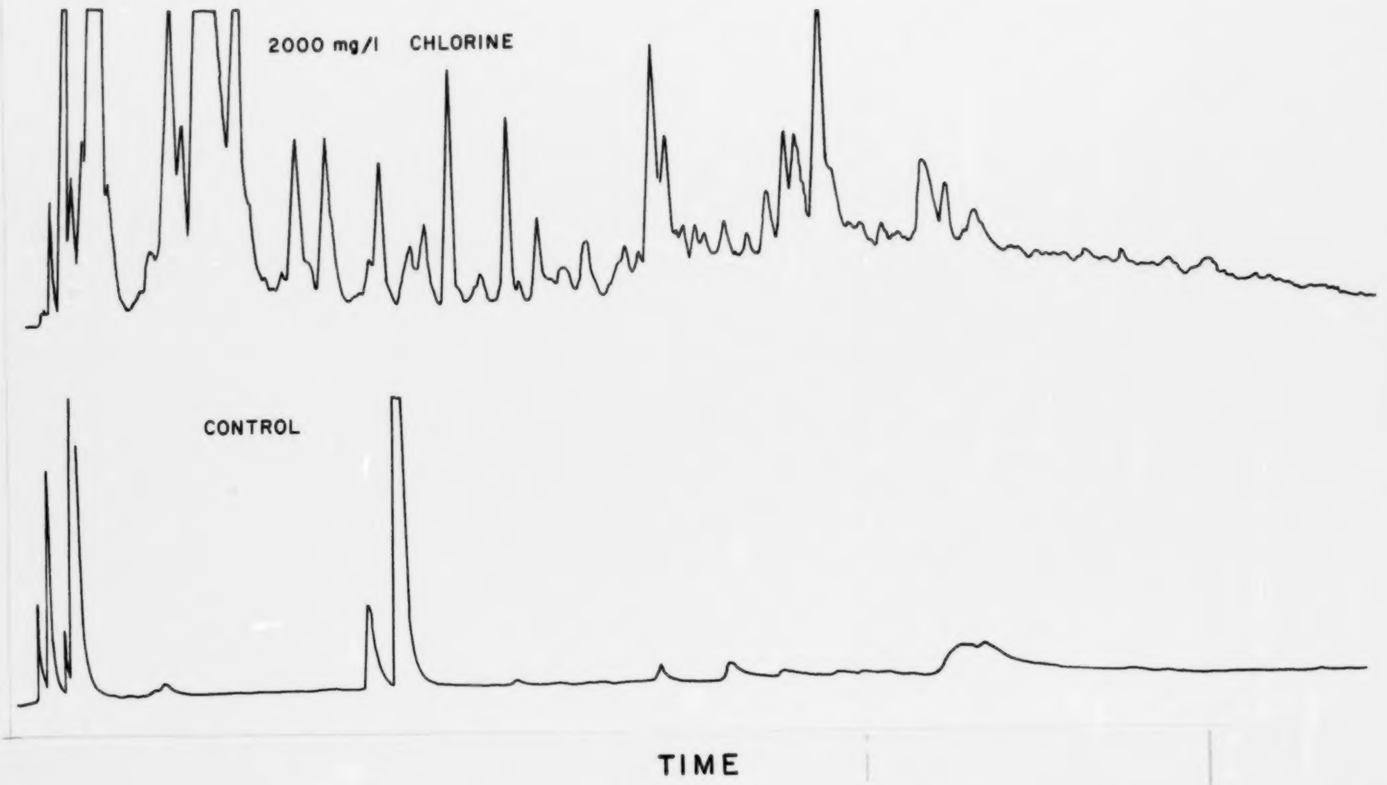

Figure 13. Gas chromatograms (Coulson electrolytic conductivity detector, halogen mode, X 4) on Denton, Texas wastewater extract. Bottom before; top, after 2000 mg/L chlorination for 1-hour contact time. Analytical conditions described in text.

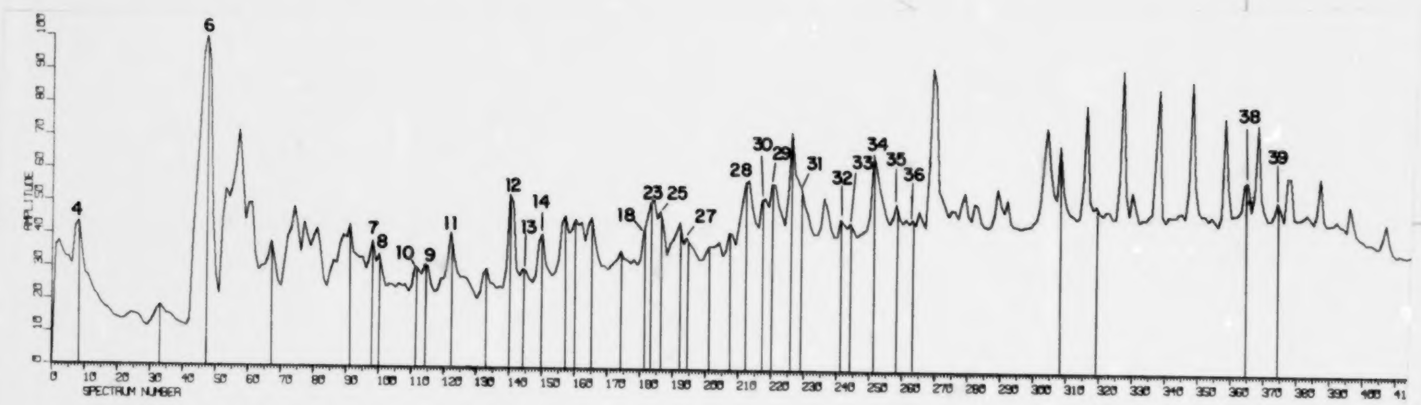

Figure 14. Reconstructed GC/MS chromatogram of Denton, Texas wastewater extract after 2000 ppm chlorine treatment for one hour. Vertical line markers show the positions of new chlorinated organics not present in control samples. Peak numbers correspond to compounds identified in Table 7.

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TABLE 7. SUMMARY OF NEW CHLORINATED ORGANICS FOUND IN "SUPERCHLORINATED" MUNICIPAL WASTEWATER

Compound Number	Compound Name[a]	Identification Status	Concentration[b] µg/l
1	Chloroform	f,g	-
2	Dibromochloromethane	f,g	-
3	Dichlorobutane	d,g	27
4	3-chloro-2-methylbut-1-ene	f	285
5	Chlorocyclohexane (118)	d,g	20
6	Chloroalkyl acetate	d	-
7	o-Dichlorobenzene	f	10
8	p-Dichlorobenzene	f	10
9	Chloroethylbenzene	e	21
10	Tetrachloroacetone	e	11
11	Pentachloroacetone	f	30
12	Hexachloroacetone	f	30
13	Trichlorobenzene	f	-
14	Dichloroethylbenzene	f	20
15	Chlorocumene (154)	d,g	-
16	N-methyl-trichloroaniline (209)	d,g	10
17	Dichlorotoluene	e,g	-
18	Trichlorophenol	e	-
19	Chloro-α-methyl benzyl alcohol	e,g	-
20	Dichloromethoxytoluene	e,g	32
21	Trichloromethylstyrene (220)	d,g	10
22	Trichloroethylbenzene (208)	d,g	12
23	Dichloro-α-methyl benzyl alcohol (190)	d	10
24	Dichloro-bis(ethoxy)benzene (220)	d,g	30
25	Dichloro-α-methyl benzyl alcohol (190)	d	-

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(TABLE 7.--Continued)

Compound Number	Compound Name[a]	Identification Status	Concentration[b] µg/l
26	Trichloro-N-methylanisole	e,g	-
27	Trichloro-α-methyl benzyl alcohol	e	25
28	Trichloro-α-methyl benzyl alcohol	e	25
29	Tetrachlorophenol	f	30
30	Trichloro-α-methyl benzyl alcohol	e	50
31	Trichlorocumene (222)	d	-
32	Tetrachloroethylstyrene (268)	d	-
33	Trichlorodimethoxybenzene (240)	d	-
34	Tetrachloromethoxytoluene (258)	d	4-
35	Dichloroaniline derivative (205)	c	13
36	Dichloroaromatic derivative (249)	c	15
37	Dichloroacetate derivative (203)	c,g	20
38	Trichlorophthalate derivative (296)	c	-
39	Tetrachlorophthalate derivative (340)	c	-

[a] Compounds may be listed more than once if GC retention times indicate distinct positional isomers.

[b] Quantitative values should only be considered as estimates since response factors and recovery data are not available for our extraction system.

[c] Mass spectral information too incomplete to propose astructure; probable molecular weight indicated in parentheses.

[d] Fragmentation pattern tentatively suggests proposed compound; probable molecular weight indicated in parentheses.

[e] Probable identification based on mass spectral interpretation.

[f] Completed identification based on MS interpretation and confirmed by comparison with a reference spectrum.

[g] Identified in runs other than 11-12-74 (Figure 14).

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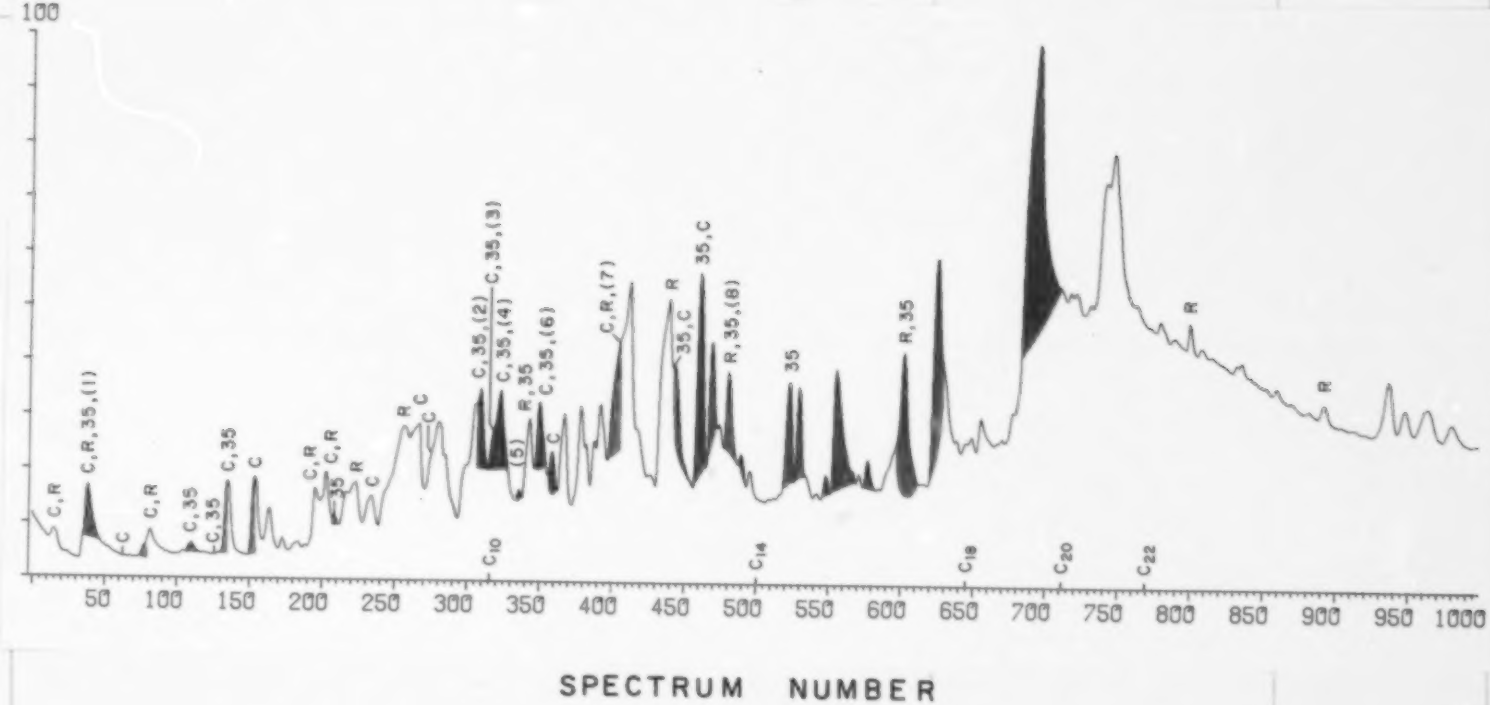

Figure 15. Reconstructed GC/MS chromatogram of superchlorinated septage extract (Reference Table 8 and text).

with an "R". Compounds identified by a priori interpretation of their mass spectra, or comparison with standard reference tables, are numbered in Figure 15 and listed in Table 8. Numerous spectra indicate chlorine isotopic clusters but cannot be identified. Also shown in Figure 15 are notations of the retention times of n-hydrocarbons C_{10}, C_{14}, C_{18}, C_{20}, C_{22}, and C_{26}, in order to give one a sense of the temperature program used. The program was essentially the same as the one described previously in this section.

Purge and trap (P/T) analysis of the septage supernatant was also conducted, using a modified P/T apparatus in conjunction with the GC/MS system. Figure 16 shows the chromatogram of a superchlorinated sample using this method. The principal features of the purge and trap modification are the use of a long, narrow tube to contain the water sample (ca. 20 ml) during purging, and the substitution of Chromosorb 102 as the GC column material. Figure 17 shows a six-compound standard used to evaluate the procedure.

TABLE 8. COMPOUNDS IDENTIFIED IN VENTURA, CALIFORNIA, SUPERCHLORINATED SEPTAGE SUPERNATANT, XAD-DIETHYL ETHER EXTRACT (REF. FIGURE 15)

Code	Compound	Spectrum Number	µg/L
(1)	Chloroform	40	(a)
(2)	Dichlorobenzene	310	67
(3)	Tetrachloroacetone[b]	317	317
(4)	Dichlorobenzene	324	179
(5)	Chlorobutandione[b]	337	-
(6)	Pentachloroacetone	348	95
(7)	Dichlorotoluene	405	24
(8)	Trichlorotoluene	484	37

[a] Quantification not attempted due to losses in workup.

[b] Tentative identification.

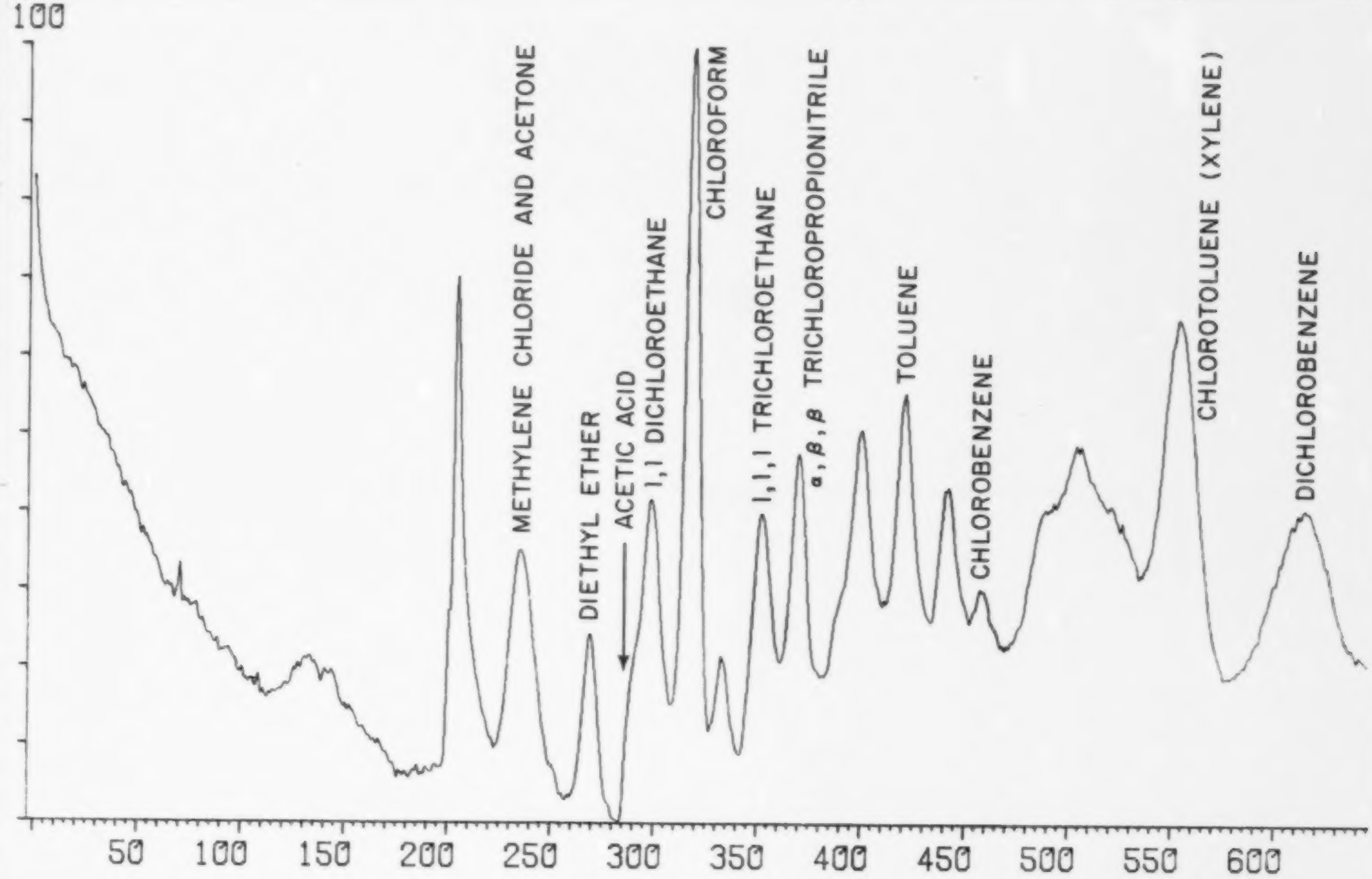

Figure 16. Reconstructed GC/MS chromatogram of purgeable organic compounds in superchlorinated septage.

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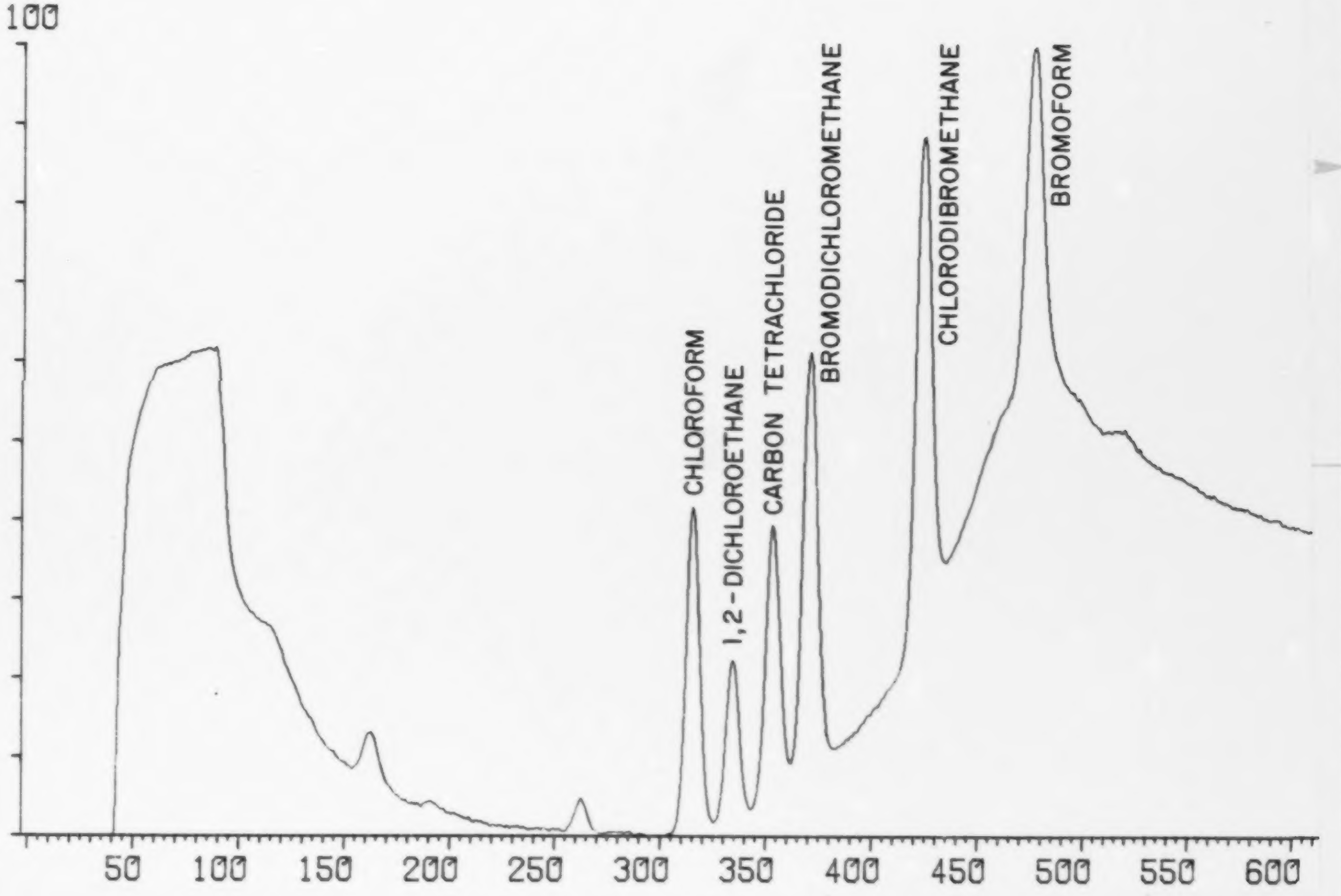

Figure 17. Reconstructed GC/MS chromatogram of purgeable reference compounds.

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LCS Analysis of GC/MS Data--

The computerized analysis of the data shown in Figure 15 was hindered by the poor quality of the raw spectra. This was partly due to the complexity of the sample matrix and partly due to a basic shortcoming of the Finnigan 6000 data system. The system acquires data for each mass using a single, fixed interpretation to allow reasonable precision when measuring a peak of low absolute intensity. The fixed time period limits the dynamic range of the mass spectral peak intensities which the spectrometer can record before reaching a saturation point. This in turn limits the range of analyte concentrations which will produce accurate representative spectra. The concentrations of analytes in the CALCLI sample cover a range of at least four orders of magnitude. This clearly exceeds the dynamic range of the data system.

One way to get usable spectra for all the analytes in such a sample is to make several GC/MS runs of the same sample at different dilutions. Mass spectra of analytes of high concentrations can be selected from the GC/MS run of the diluted sample; mass spectra of the trace components can be produced from the GC/MS run of the sample at higher concentration. Another alternative is to concentrate the sample until spectra for the smallest GC peaks are interpretable, and then select spectra from the sides of GC peaks whose apical spectra contain saturated mass spectral peaks. Thus, the analyst can adjust for the saturation problem of the larger GC peaks by the proper selection of the representative mass spectrum. In situations where the quantity of sample is limited, the latter procedure is preferable, and this was the procedure which was used to produce the CALCLI data set.

Unfortunately, the gross distortion of some mass spectra due to saturated mass spectral peaks can cause problems for a computerized data analysis system which is forced to analyze all spectra as though each were produced within the dynamic range of the instrument. Nevertheless, the data were analyzed using the LCS computer program to search for ion fragments containing two, three, and four chlorines. For all runs the precision estimate parameter was 0.1, and the variation estimate parameter was -50.

Figure 18 shows LCS chromatograms for the portion of Figure 15 between spectrum numbers 290 and 510 (referred to hereafter as the CALSTI data set). It is seen that the total ion chromatogram is simplified by the limited cluster search procedure. In the LCS for two chlorine atoms (middle trace) one sees a resolution of the overlapping peaks in the 300-330 scan number region, the simplification of the region around 350, and the confirmation of halogen content in the region between 450 and 490.

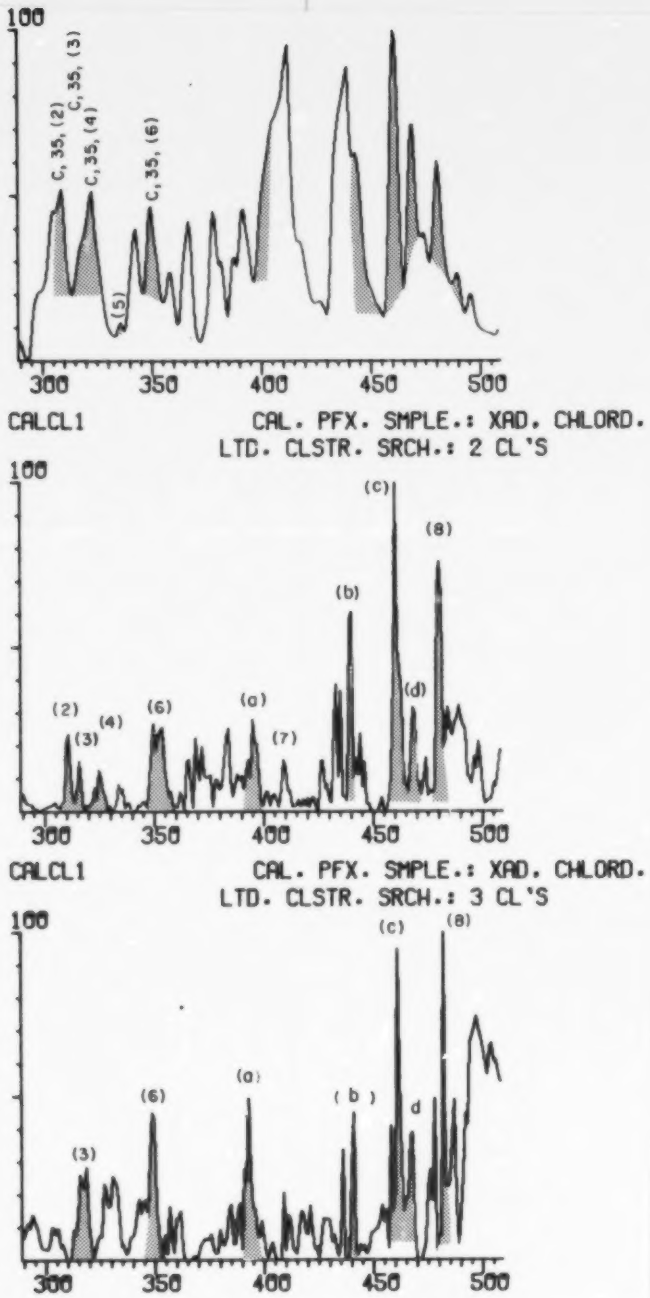

Figure 18. Total ion chromatogram (top) and LCS chromatograms for two chlorines (middle) and three chlorines (bottom) for CALSTI data set.

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Note that (3), (6) and (8), tetrachloroacetone, pentachloroacetone and trichlorotoluene, are intense in the trichloro- and dichloro- cluster searches, while dichlorobenzene (2) isomer fades in the trichloro-search. Two other dichloro-compounds (4) and (7) appear also in the trichloro-search but an analysis of the TIC suggests that overlapping, polyhalogenated peaks may be present. In the high scan number region, peaks (a), (b), (c) and (d), not identified as yet, probably contain at least three halogen atoms each.

Further analysis of the CALSTI data set has shown that data interpretation assisted by LCS increases the number of halogen-containing spectra identified for every halogen combination tried. While the LCS program does not give a dramatic simplication of the TIC chromatogram, it would seem to be helpful in identifying peaks with halogen content for further study. A more detailed analysis of the characteristics of the program suggest that further refinements may improve its usefulness for the analysis of complex mixtures for chlorinated organics.

Microcoulometric Analysis of XAD-Resin/Ether Extracts--

The ether XAD-2 extracts used in the GC/MS analysis were also analyzed by a pyrolysis/microcoulometric procedure (71) which converts organic halogen to halide ion which is titrated. The detected value for the raw septage supernatant was 85 µg/L as chloride. After superchlorination the supernatant showed a value of 870 µg/L. Given the fact that the XAD procedure does not quantitatively adsorb or desorb all of the organics in the sample, the observed value indicates a high level of organic halogen in the chlorinated supernatant. A similar procedure was applied to a sample of combined sludges from an east coast waste treatment plant. The supernatant of the sludges showed an organohalogen level by the XAD/ether method of 39±4 µg/L (two determinations). After two and four hours of contact time with a high level of chlorine (>1000 µg/L), the sludge supernatant showed values of 414±1 and 507±4 µg/L, as chloride.

These data illustrate that total organically bound halogen is often much higher than the sum of volatile organohalides which may be determined by GC (69, 70). Moreover, the high levels suggest that caution should be used in the disposal of "superchlorinated" sludges and other waste products, particularly when leaching into ground water may be possible.

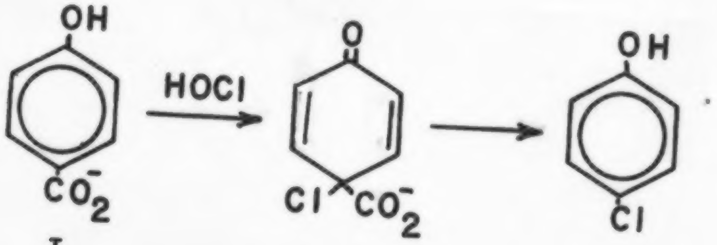
Interpretation of Volatile Chlorinated Organics Formed from Superchlorination of Wastewater Products--

It is now well established that chlorination of wastewaters and other municipal waste products produces new chlorinated organic compounds. Tables 7 and 8 list some of these compounds; others are listed by Glaze _et al_. (36, 60, 75) and Jolley

(9, 61, 62), but many others remain unidentified at this time. The precursors of these new substances and their effects on the environment (including man) are unknown at this time. The former may prove more difficult to determine, primarily because of our ignorance of the molecular types present in municipal wastes. Undoubtedly, these wastes are extremely complex and one may never know with surety the source of the new chlorinated compounds that are listed in Table 2 and in the other references mentioned.

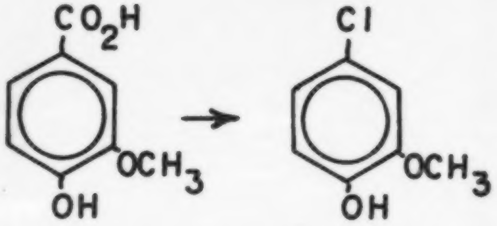
It is striking to note, however, that many of these newly formed halocarbons are similar to those found from laboratory chlorination of humic and fulvic acid (51, 52, 53, 54). However, in the case of municipal wastewater and septage, the subjects studied in this project, one would not expect humic substances to be major components. That the by-products observed in this work are structurally similar and in many cases identical to the compounds found by Rook and by Christman *et al*. from the chlorination of humic material and surface water organics may not indicate identical precursors. Rather, they may simply reflect the fact that similar organic structural units are found throughout the biological world.

Three types of compounds listed in Table 7 deserve special mention: the polychlorinated acetones, 3-chloro-2-methybut-1-ene, and several chlorinated alkyl benzenes. The latter include chlorinated benzene, toluene, ethylbenzene, and cumene isomers. Direct chlorination of the corresponding aromatic compounds may be possible, but is unlikely since some type of activating substituent is usually required for facile chlorination in aqueous systems (10). More likely, these neutral chloroaromatics result from the decarboxylation of the corresponding aromatic acids that either are originally present in the waste product or are formed during the oxidation/chlorination process. It is not clear whether chlorination occurs before or after oxidative degradation; however, Larson and Rockwell (93) have shown that chlorination of p-hydroxybenzoic acid (I) gave 4-chlorophenol:



I

The absence of phenol, 2-chlorophenol, or 2,6-dichlorophenol suggested to the authors a two-step process with chlorination as the initial step. Vanillic acid (II) yielded 4-chloro-2-methoxylpheno (III), apparently by a similar process (93). Recently, Sievers and co-workers (94) have observed the formation of toluene and several other aromatic hydrocarbons from the chlorination of municipal wastewater, and saturated aliphatic hydrocarbons from the ozonation of the same water. The latter have been shown to occur as the result of cleavage and decarboxylation of oleic acid (95).



II

III

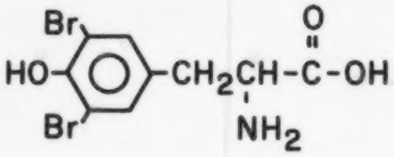
The major product observed in this work, 3-chloro-2-methylbut-1-ene, may be related to the chloroisopentanol observed by Rook (51). Both are isoprenoids and most probably result from the degradation of some aliphatic component of a humic-like polymer. Likewise, the polychlorinated acetone derivatives may arise from a cleavage of aliphatic side chains on the polymer before or after partial chlorination.

SECTION 6

CHLORINATION OF AMINO ACIDS IN MUNICIPAL WASTE PRODUCTS

INTRODUCTION

The purpose of the experimental investigation described in this section is to determine to what extent chlorinated compounds are formed during the aqueous chlorination of amino acids in municipal waste treatment plants. Chlorination of amino acids through the use of various chlorination agents has been studied by several previous workers (96-110), but no documentation currently exists of these reactions in the treatment of waste products which may contain amino acids or polypeptides. The relevance of this research may be inferred from recent toxicological data on one halogenated amino acid, namely, 3,5-dibromotyrosine (I).



I

Fortunato 111 has shown that this compound inhibits the initial synthesis of thyroid hormones from inorganic iodide with resulting effects on the hormone concentration in both the thyroid and vascular spaces. One may postulate detrimental effects from other halogenated amino acids which may be formed in water treatment and discharged into the environment for possible consumption by man or other species.

This work has examined analytical methods for the identification of amino acids in aqueous solution, and has applied these methods to the determination of amino acids and their chlorination products in chlorinated municipal waste products. Particular attention has been given to a study of

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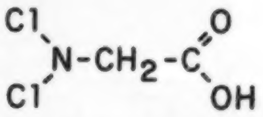
"superchlorinated" sewage sludges and other products, it being assumed these extreme conditions would favor the formation of new halogenated products. Superchlorination as used in this context means the use of high concentrations of chlorine (0.2-0.4%) for the oxidative stabilization of sludges and other sewage products.

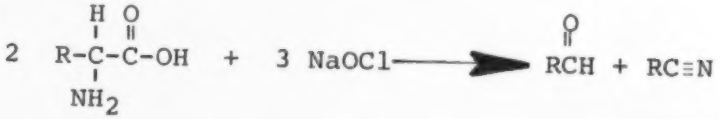
SUMMARY OF PREVIOUS WORK

Langheld (96) allowed α-amino acids to react with sodium hypochlorite solutions and found that an aldehyde, carbon monoxide, and ammonia were produced. Dakin (97) showed if one equivalent of the N-chloro compound, chloramine-T [N-chloro-o-toluene-sulfonamido) sodium] was allowed to react with α-amino acids, the corresponding aldehydes were produced; and if two equivalents of the reagent were used (99) the corresponding nitrile resulted. Wright (100) showed that sodium hypochlorite when reacted with glycine produced complete chlorination of the amino group, forming dichloroamino acetic acid (II).



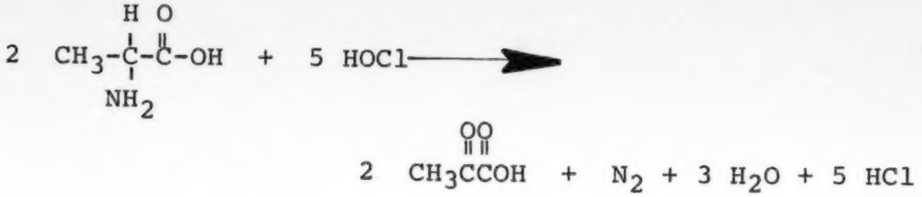
II

Norman (101) studied the action of sodium hypochlorite on glycine and expressed doubt as to the formation of chlorinated intermediates in the oxidation of glycine by hypochorite; however, Wright (102) provided further evidence for the formation of chloroamino derivatives as intermediate products from the oxidation of amino acids by hypochlorites except in extreme alkaline solutions. He also found that aldehydes were formed from several amino acids when reacted with sodium hypochorite and that nitriles formed as the predominant product at lower pH values:



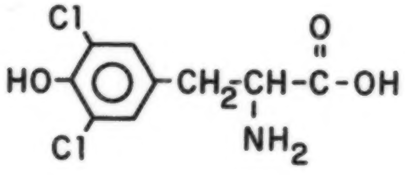
$$2\ \ R-\overset{H}{\underset{NH_2}{C}}-\overset{O}{\overset{\|}{C}}-OH\ +\ 3\ NaOCl \longrightarrow R\overset{O}{\overset{\|}{C}}H + RC\equiv N \qquad (1)$$

Ingols (103-4) stated that when hypochlorous acid reacts with alanine, pyruvic acid is formed by oxidative deamination:



$$2\ CH_3-\underset{NH_2}{\overset{H}{C}}-\overset{O}{\overset{\|}{C}}-OH + 5\ HOCl \longrightarrow 2\ CH_3\overset{O}{\overset{\|}{C}}\overset{O}{\overset{\|}{C}}OH + N_2 + 3\ H_2O + 5\ HCl \qquad (2)$$

Aleksiev (105) showed that 3,5-dichlorotyrosine (III) is produced when L-leucyl-L-tyrosine or the hormone oxytocin reacts in 85% formic acid with chlorine saturated carbon tetrachloride at -20°C. However, no spectroscopic confirmatory data was given. Kantouch and co-workers (106) allowed sodium hypochlorite to react with a few α-amino acids and found that most of the amino acids reacted quickly causing oxidation and/or formation of chloro derivatives. At pH 2, substitution occurred mainly by forming the N-chloro and N-dichloro derivatives. By acylation of the amino group, the oxidation rate was reduced.



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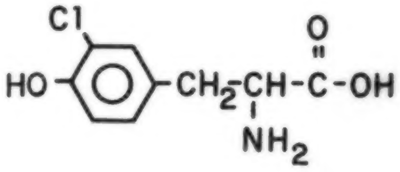

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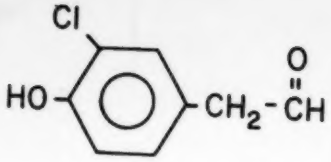
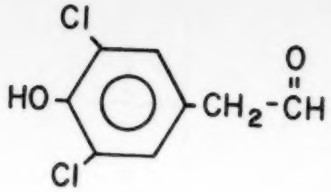
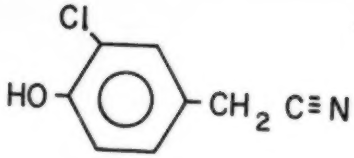
It was stated that 3-chloro (IV) and 3,5-dichlorotyrosine (III) were produced from tyrosine at pH 2 and could be detected by two dimensional paper chromatography, but no spectroscopic evidence was cited as proof of structure. Pereira and co-workers (107) studied the reaction of hypochlorous acid with several α-amino acids and found that L-phenylalanine, reacted with hypochlorous acid to produce phenylacetaldehyde and phenylacetonitrile. Glutamic acid reacted with hypochlorous acid to produce β-aldehydopropionic acid. Cysteine reacted with either one or two equivalents of hypochlorous acid at room temperature to produce cysteic acid or cystine. L-tyrosine produced 3-chloro (V) and 3,5-dichloroaldehyde (VI) along with the 3-chloro (VII) and 3,5-dichloronitrile (VIII).



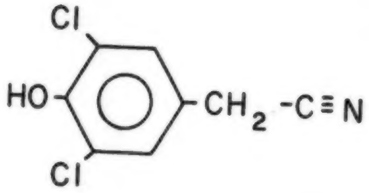
V



VI



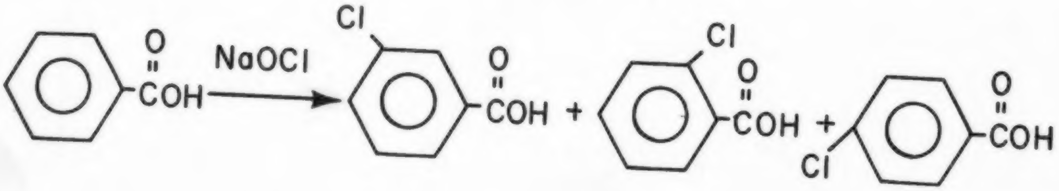
VII



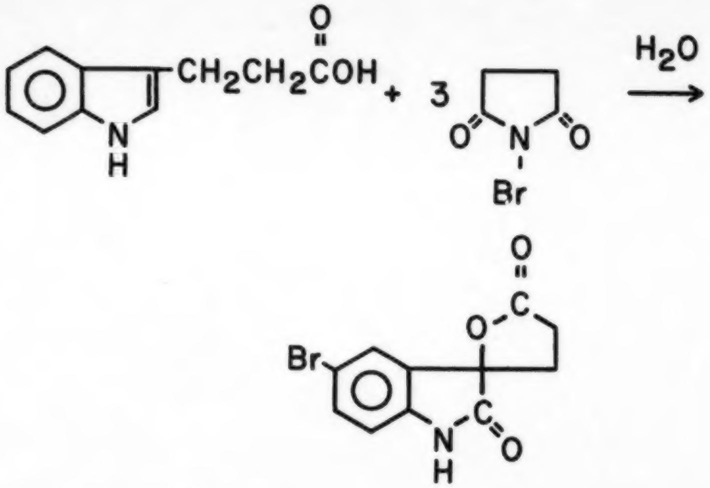
VIII

It should be noted that no evidence for the formation of 3-chloro and 3,5-dichlorotyrosine was reported in this paper. Chlorination of dipeptides gave the corresponding N,N-dichloropeptides, with no cleavage of the amide bond reported.

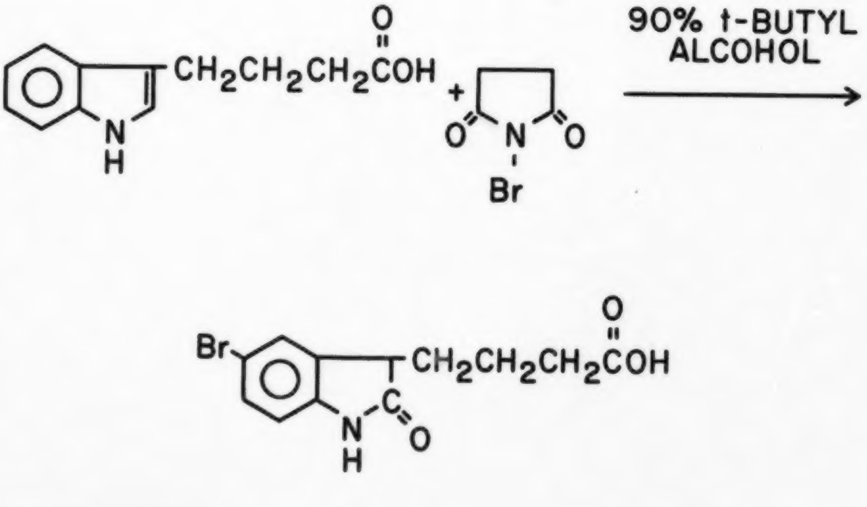
Halogenation of compounds similar to the aromatic amino acids have been studied as models of the type of reactions to be expected with the aromatic amino acids. Smith (112) reacted aqueous sodium hypochlorite with benzoic acid and obtained a mixture of three isomeric monochlorobenzoic acids with some dichloro acid:



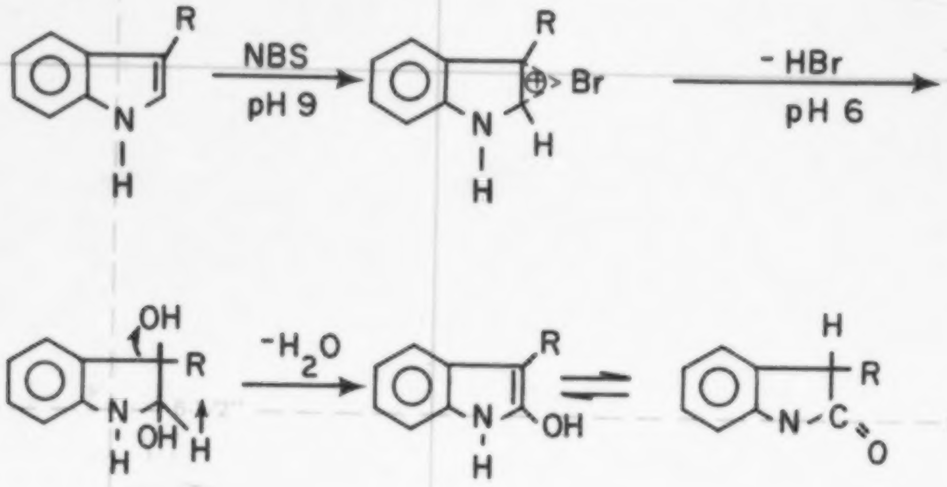
Hopkins and Chisholm (113) stated that substances in which the orientation of substituent groups is most favorable give monochloro derivatives in almost theoretical yield when they react with aqueous sodium hypochlorite. These substances include vanillin, anisic acid, and piperonylic acid. Morton (114) stated that chlorination by mechanisms similar to aromatic substitution seems to occur with pyroles and indoles. Lawson and coworkers (115) found that indole-3-propionic acid reacted with three moles of N-bromosuccinimide (NBS) in aqueous media to produce spirolactone dioxindole-3-propionic acid with a bromine in the 5 position:


CH2CH2COH
3
H2O
Br

Hinman and Bauman (116) found when indole-3-butyric acid was reacted with N-bromosuccinimide in 90% t-butyl alcohol and 10% water containing no added acid or base, 5-bromooxindole-3-butyric acid was obtained in 73% yield.


CH2CH2CH2COH
90% t-BUTYL ALCOHOL
Br

The addition of water to the reaction mixture apparently is responsible for benzene ring bromination of indole. Green and Witkop (117) proposed the following mechanism for the formation of oxindole with N-bromosuccinimide (NBS):



Haberfield and Paul (118) reported evidence that N-chloro-N-methylaniline is the intermediate in the chlorination of N-methylaniline by calcium hypochlorite in carbon tetrachloride solvent. The infrared spectrum of the reaction mixture shows a loss of the N-H stretching band. Analysis of a sample which had been treated with potassium iodide and sodium thiosulfate revealed the principal product to be unreacted N-methylaniline. With omission of the potassium iodide, the principal products were found to be o-chloroaniline, p-chloroaniline, and some dichloroaniline.

Amino Acids in Treated and Untreated Sewage

Sastry and co-workers (119) examined sewage effluents from chemical clarification, mechanical and biological filtration, septic tanks, activated sludge process and from natural purification of flowing sewage obtained from the Indian Institute of Science sewage works at Bangalore, India and from the city of Bangalore. Using a circular paper chromatographic technique for the analysis of the amino acids, their work showed that raw sewage contained practically all the essential amino acids. Raw sewage obtained from the sewage works at the Institute was found to have 0.36 to 1.01 milligrams of free amino acids and 65.1 to 91.3 milligrams of total amino acids per gram of solid. Tryptophan was not present in the free form and acid hydrolysis destroyed it in the solid material. The effluents that were btained by chemical clarification using alum and mechanical filtration were found to contain considerable amounts of amino acids.

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Almost all the amino acids from Bangalore sewage were completely removed as it ran over a short distance of 1.29 miles along a natural channel, presumably due to the presence of bacteria in the channel. The effluents from the activated sludge process were also found to be almost free from amino acids.

Painter and Viney (120) indicated that free amino acids were about 16% of the total amino acids in whole sewage. Work by Subrahmanyam and co-workers (121) showed that the activated sludge method of sewage purification removed nearly all of the amino acids. Raw sewage was reported to contain 86 milligrams of amino acids per gram of dried solids and after four hours of aeration only a trace was found. Six hours of aeration gave no detectable amino acids. Cystine, lysine, histidine, and arginine were said to be present only in trace amounts in the raw sewage, and no proline was found. Kahn and Wayman (122) obtained raw sewage and sewage effluents from Denver, Colorado wastewater treatment plant which had only primary treatment facilities. Other samples were obtained from three other cities each of which had some form of the activated sludge process. The total amino acids found by two-dimensional chromatography are presented in Table 9. Phenylalanine, tyrosine, and tryptophan were not found in any of the samples. Hunter and Heukelekian (123) showed that wastewater from Highland Park, New Jersey contained a particulate fraction which contained 19% amino acids. Hanson and Lee (124) indicated that nearly all the amino acids in domestic wastewater are present in the combined state, in disagreement with Kahn and Wayman (122). Hunter (125) compiled the known information about the occurrence of amino acids in untreated domestic wastewater; his compilation is presented in Table 10.

TABLE 9. TOTAL AMINO ACIDS FOUND IN THE SEWAGE SAMPLES FROM FOUR UNITED STATES CITIES (122)

Total Free Amino Acids in Raw Denver Sewage:	115 mg/l
Total Combined Amino Acids in Raw Denver Sewage:	165 mg/l
Total Free Amino Acids in Denver Primary Effluent:	30 mg/l
Total Combined Amino Acids in Denver Primary Effluent:	35 mg/l
Total Combined Amino Acids in Chicago Activated Sludge Effluent:	10 mg/l
Total Combined Amino Acids in Trenton Activated Sludge Effluent:	5 mg/l
Total Combined Amino Acids in Hamilton Township, New Jersey, Activated Sludge Effluent:	5 mg/l

66

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TABLE 10. AMINO ACID CONTENT OF THE SOLUBLE FRACTION IN UNTREATED DOMESTIC WASTEWATER (125)

Amino Acid	Concentration (mg/l)
Cystine	0 - Trace
Lysine and Histidine	Trace
Histidine	Present
Lysine	Absent
Arginine	Trace
Serine, glycine, and aspartic acid	0.02 - 0.13
Threonine and glutamic acid	0.01 - 0.18
Alanine	0.02 - 0.09
Proline	0
Tyrosine	0.06 - 0.09
Methionine and valine	0.05 - 0.24
Phenylalanine	0.02 - 0.33
Leucine	0.06 - 0.28
Tryptophan	Present

Gas Chromatography of Amino Acids

The identification and quantification of amino acids by gas chromatography has been the subject of numerous papers over the past several years. A search of the literature indicates that the N(O)-trifluoroacetyl-alkyl esters, N(O)-heptafluoro-alkyl esters, and the trimethylsilyl (TMS) amino acid derivatives have received the most attention.

Stalling and co-workers (126) synthesized bis(trimethylsilyl) trifluoroacetamide (BSTFA) for the silylation of amino acids, since previous silylation reagents interfered with the separation of glycine and alanine. Their work with BSTFA showed that it

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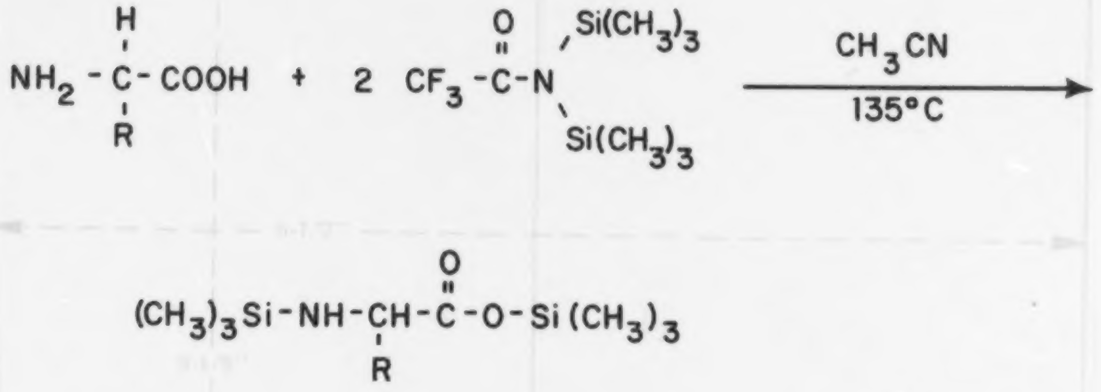
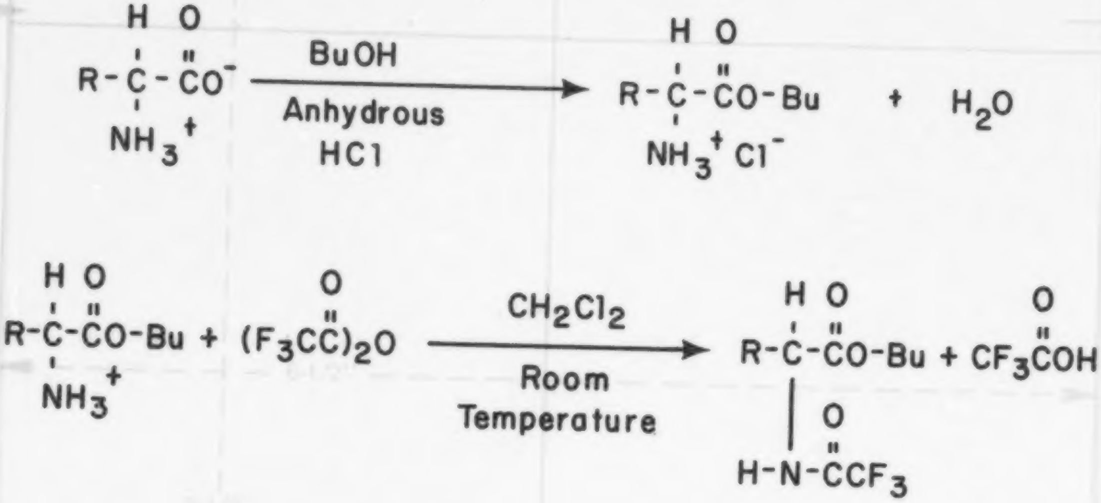
had increased volatility and appeared with the solvent front, and had greater solubility in some solvents than former silylation reagents such as bis(trimethylsilyl) acetamide. The fluorine in BSTFA resulted in less silica deposits and thus decreased detector noise. No interference occurred with glycine and alanine; however, no reproducible chromatographic peaks could be obtained for arginine. Moreover, several of the amino acids gave more than one trimethylsilyl derivative. The following reaction is a typical derivatization of amino acids using BSTFA:



$$NH_2-\underset{R}{\overset{H}{C}}-COOH + 2\ CF_3-\overset{O}{\overset{\|}{C}}-N(Si(CH_3)_3)_2 \xrightarrow[135°C]{CH_3CN} (CH_3)_3Si-NH-\underset{R}{CH}-\overset{O}{\overset{\|}{C}}-O-Si(CH_3)_3$$

Gehrke and co-workers (127) made a study of the BSTFA derivatives of the twenty protein amino acids and emphasized the chromatographic separation of the derivatives, as well as the precision and accuracy of the method. Attention also was given to silylation as a function of time, reaction temperature and stability of the derivatives. As indicated above, several of the amino acids, including glycine, histidine, arginine, lysine, and tryptophan gave more than one trimethylsilyl derivative. The N-(O)-trimethyl silyl (TMS) amino acid esters were found to be completely stable for a period of five to seven days when stored at room temperature in a tightly capped vial. Glycine was the only exception and deteriorated in three hours. Gehrke and Leimer (128) reported the silylation and resolution of the twenty protein amino acids on a single 10% w/w OV-11 column using BSTFA with acetonitrile as the solvent.

One of the earliest works with N-substituted ester derivatives of amino acids for gas chromatography was by Young (129), who reported the N-acetyl-n-butyl esters of glycine, alanine, valine, leucine, isoleucine, and proline. Lamkin and Gehrke (130) studied the N(O)-trifluoroacetyl-n-butyl esters and the N(O)-TFA-methyl esters of the amino acids. They found that serious losses were involved in concentrating the N(O)-TFA-methyl esters. Single chromatographic peaks were obtained for all the common protein amino acids as the N(O)-TFA-n-butyl esters except for tryptophan and arginine. Tryptophan gave two peaks and could not be converted into a single peak with longer acylation. The esterification and acylation reactions were given as follows:



$$R-\underset{\underset{NH_3^+}{|}}{\overset{\overset{H}{|}}{C}}-\overset{\overset{O}{\|}}{C}O^- \xrightarrow[\text{Anhydrous HCl}]{\text{BuOH}} R-\underset{\underset{NH_3^+\,Cl^-}{|}}{\overset{\overset{H}{|}}{C}}-\overset{\overset{O}{\|}}{C}O-Bu \;+\; H_2O$$

$$R-\underset{\underset{NH_3^+}{|}}{\overset{\overset{H}{|}}{C}}-\overset{\overset{O}{\|}}{C}O-Bu + (F_3\overset{\overset{O}{\|}}{C}C)_2O \xrightarrow[\text{Room Temperature}]{CH_2Cl_2} R-\underset{\underset{H-N-\overset{\overset{O}{\|}}{C}CF_3}{|}}{\overset{\overset{H}{|}}{C}}-\overset{\overset{O}{\|}}{C}O-Bu + CF_3\overset{\overset{O}{\|}}{C}OH$$

Coulter and Hann (131) prepared the N-acetyl-n-propyl esters of the amino acids. They stated the hydrochloride of histidine had to be neutralized using lithium or sodium carbonate before it could be acylated. Arginine had to be converted to ornithine while histidine was either converted to aspartic acid or the hydrochloride neutralized with lithium carbonate. Their procedure required two GC columns for separation.

Zumwalt and co-workers (132) reported work using the N(O)-TFA-n-butyl esters of the amino acids for the quantitative analysis of the amino acids in complex biological substances. Cation or anion exchange cleanup procedures were used before derivatization. Their work also reported an optimum molar ration of 50:1 (trifluoroacetic anhydride to amino acid) maximizes reproducibility of acylation, stability of the derivatives and maintenance of small sample volume. Two columns were again required for separation of the derivatized amino acids. Gehrke and co-workers (133) reported a dual column system in which histidine, arginine, tryptophan, and cystine would elute quantitatively and resolve as single peaks from the other N(O)-TFA-n-butyl esters. They observed strong substrate-derivative interaction with the diacyl histidine derivative when using OV-120 columns.

Moss and Lambert (134) reported the GLC separation of twenty protein amino acids on a single column using the N(O)-heptafluorobutyryl-n-propyl ester derivatives. Their work showed that the mono-heptafluorobutyryl-n-propyl derivative of histidine could be converted to a diacyl derivative if acetic anhydride was injected simultaneously with the sample into the gas chromatograph. This resulted in a sharp single peak for histidine rather than a broad peak for the monacyl derivative.

Hardy and Kerrin (135) prepared the trimethylsilyl-n-butyl esters of the amino acids and reported that these derivatives could be separated in less than 35 minutes on a lightly loaded texture glass GC column. They also found that these derivatives gave the best separation when acetonitrile was used as the solvent for trimethylsilylation. Close examination of their chromatogram showed numerous small peaks which correspond in retention time to the fully trimethylsilylated amino acid derivatives, suggesting that esterification of the amino acids was not complete.

Zanetta and Vincendon (136) reported the preparation of N(O)-heptafluorobutyryl-isoamyl esters of amino acids and separation on a single column. Jonsson and co-workers (137) studied the N(O)-HFB-*n*-propyl derivatives with emphasis on variation of the esterification, acylation, and chromatographic procedures. They determined the relative molar response, linearity of response and the stability of the derivatized samples. Their findings showed that the two step esterification and acylation procedure of Moss and Lambert (134) gave the best results. Histidine and lysine were shown to require two esterification periods since they have very low solubility in high concentrations of HCl. The amino acid derivatives were all found to be stable for at least three days.

The use of the mass spectrometer as a highly specific and versatile detector for the gas chromatograph has been documented by a large number of workers. Among others, Oro and co-workers (138) have utilized GC/MS for the analysis of derivatized amino acids. The principal advantage of this method is that it allows for specific confirmation of an analysis based on computerized, mass specific techniques such as multiple ion detection (139). Thus, GC/MS is particularly suited for analyses in complex matrices such as biological and environmental samples.

EXPERIMENTAL PROCEDURES

Materials and Instrumentation

Purified Water

The water used in this work was prepared from the tap by passage through a Continental deionizer unit (Model 200), a

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Calgon filtrasorb 400 activated carbon column and then through a Whatman qualitative filter.

Solvents

Ethyl acetate was purchased from Pierce Chemical Company, Rockford, Illinois. Acetonitrile - (Baker Analyzed reagent) was purchased from J. T. Baker, Philadelphia.

Amino Acids

Amino acid standards were obtained from Jack Graff Associates, Santa Clara, California.

Other Reagents

Heptafluorobutyric anhydride was purchased from Pierce Chemical Company, Rockford, Illinois. Cupric chloride was obtained from Matheson, Coleman and Bell, Norwood, Ohio. Dry HCl gas was purchased from Union Carbide. Anhydrous sodium sulfite (Analytical Reagent grade, Mallinckrodt) was heated for two hours at 130°C in an oven. Hydrochloric acid, ammonium hydroxide, chloroform and sodium metal were obtained from various suppliers and taken from available laboratory stocks. The *ortho*tolidine-arsenite (OTA) reagents for the residual chlorine determinations were prepared according to *Standard Methods* (140). The chlorine gas used to prepare the hypochlorous acid was purchased from Dixie Chemical Company, Houston, Texas, and was claimed by the manufacturer to contain 100% active chlorine. The *n*-propanol, *iso*amyl alcohol, methanol, and *n*-butanol were purchased from Fisher Company, Fairlawn, New Jersey and were Certified Grade. All alcohols were re-distilled in all glass apparatus after refluxing two hours over magnesium turnings, then stored under dry conditions at low temperature.

Resins

The cation exchange resin, Dowex 50W-X8 (sodium ion form, 100-200 mesh) and Chelex 100 resin (200-400 mesh) were obtained from Bio-Rad Laboratories, Richmond, California.

Gas Chromatography/Mass Spectrometry

The Finnigan Model 3200 gas chromatograph/mass spectrometer system with a Model 6100 data system was used to separate, detect, and identify the amino acid derivatives. The system was also used to separate, detect, and identify the reaction products of the amino acids with HOCl. All test mixtures and extracts were chromatographed using a 5 foot by 2 mm I.D. glass column packed with 10% SP-2100 on 100/120 Supelcoport (Supelco, Inc., Bellafonte, Pennsylvania). The data system was used to quantify the GC peaks and to separate any unresolved peaks using the limited mass search feature.

Spectrophotometry

A Coleman Model 295 spectrophotometer was used to measure the color produced in the OTA method for determining residual chlorine.

Amino Acid Analyzer

A Beckmann Model 120-C amino acid analyzer, which is located in Robert W. Gracy's laboratory in the Chemistry Department of North Texas State University, was used in this work. It was operated under standard conditions.

Glassware

All glassware was well cleaned using chromic acid cleaning solution, then rinsed with tap water, deionized water, and finally with redistilled acetone. The glassware was then placed in an oven at 130-150°C for 8 to 10 hours.

Methods of Analysis

The method used to isolate, concentrate, and analyze free amino acids in waste waters is shown in Figure 19 and is described below.

Isolation and Concentration of Amino Acids in Aqueous Solution

A procedure very similar to that used by Gardner and Lee (141) was used in this work. The procedure consists of isolation and purification of amino acids by a combined ion exchange/ligand exchange method (Figure 19).

A glass column (36 cm x 1.5 cm I.D.) with standard taper ground glass joint at the top was slurry packed with 30 cm of Dowex 50W-X8 (hydrogen ion form, 200-400 mesh) (141-143). Glass wool plugs of one centimeter length were used at both ends of the Dowex resin.

The ligand exchange column is a modification of that used by Siegol and Degens (142). A glass column (36 cm x 1.5 cm I.D.) with standard taper ground glass joint at the top and glass wool plug was slurry packed with three cm of Chelex-100-NH_3 resin. Twenty seven cm of Chelex-100-Cu-NH_3 resin was slurried on top of the Chelex-100-NH_3 resin and capped with a 1 cm glass wool plug.

Three liter separatory funnels with standard taper ground glass joints at the effluent end were used to hold the samples.

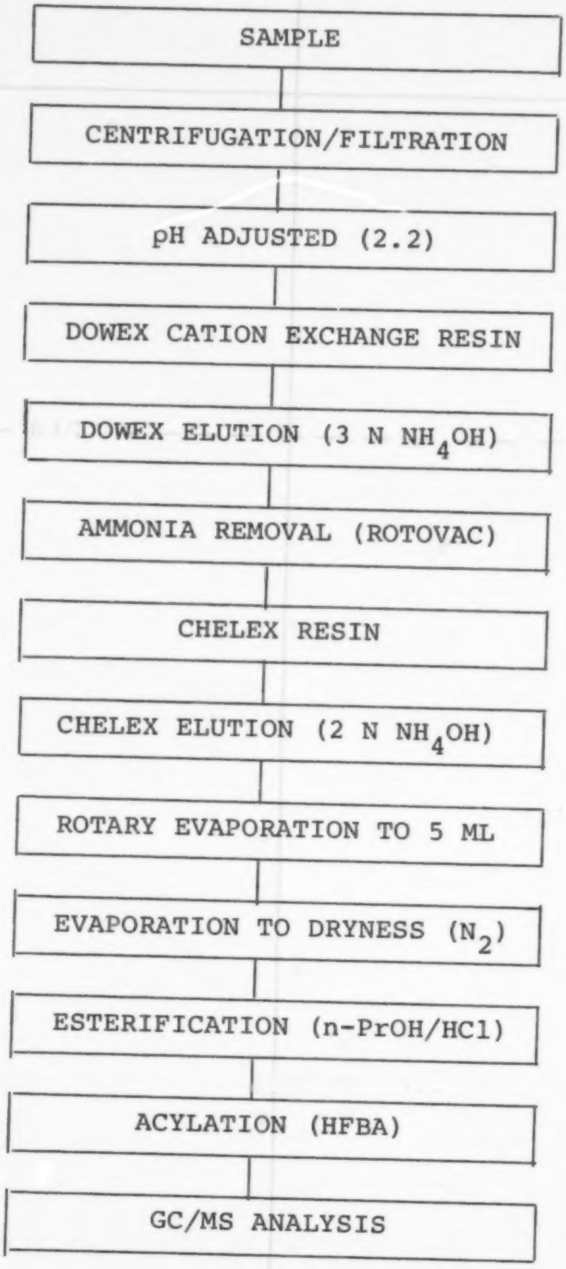
SAMPLE
CENTRIFUGATION/FILTRATION
pH ADJUSTED (2.2)
DOWEX CATION EXCHANGE RESIN
DOWEX ELUTION (3 N NH_4OH)
AMMONIA REMOVAL (ROTOVAC)
CHELEX RESIN
CHELEX ELUTION (2 N NH_4OH)
ROTARY EVAPORATION TO 5 ML
EVAPORATION TO DRYNESS (N_2)
ESTERIFICATION (n-PrOH/HCl)
ACYLATION (HFBA)
GC/MS ANALYSIS

Figure 19. Scheme for the separation of amino acids in wastewater products.

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Nitrogen pressure applied at the top of the separatory funnel was used to control the flow rate at 10 ml per minute. Wastewater samples were treated with excess sodium sulfite to quench any chlorine residual, and then the samples were centrifuged, filtered, adjusted to pH 2.2, and passed through the Dowex column. The column was eluted with 100 ml of 3N NH_4OH and the ammonia removed in a Rotovac evaporator. The concentrate was passed through the Chelex resin column. The amino acids were eluted with 100 ml of 2N ammonia solution, and ammonia was removed from the eluate by rotary evaporation at 60°C and then transferred to a 5 ml reaction vial where the drying continued at 60°C under a gentle stream of nitrogen.

Derivatization Procedure

The amino acid derivatives used in this study are the N(O)-heptafluorobutyryl-isoamyl and the N(O)-heptafluorobutyryl-n-propyl esters (134, 136, 137).

The N(O)-heptafluorobutyryl-isoamyl esters were prepared using the method of Zanetta and Vincendon (136), with some major variations. The esterification reagent was triisoamoxymethane which was prepared in the laboratory according to the procedure of Gilman (144). One ml of the esterification reagent was added for each 10 µmoles of dried amino acids in a reaction vial. Fifty µl of concentrated HCl was added and the vial capped and heated in a sand bath at 110°C for one hour. The esterification reagent was then evaporated under a stream of nitrogen at 60°C. One half milliliter of acetonitrile was then added along with 100 microliters of HFBA for each 10 µmoles of amino acid. The vial was capped and heated in a sand bath for 10 minutes at 150°C. The derivatized amino acids were dried under a gentle stream of nitrogen at room temperature, and dissolved in an appropriate volume of ethyl acetate for GC/MS analysis.

The N(O)-heptafluorobutyryl-n-propyl esters of the amino acids were prepared in a reaction vial by adding 3 ml of 8M HCl in n-propanol. The reaction vial was capped and heated in a sand bath for 10 minutes at 100°C. The esterification reagent was evaporated under a gentle stream of nitrogen. The propylation procedure was repeated and the n-propyl esters were dried under a gentle stream of nitrogen at 60°C. After drying, 1 ml of acetonitrile was added with 1 ml of HFBA. The vial was capped and heated at 150°C for ten minutes in a sand bath and then cooled to room temperature. The derivatized amino acids were then dried under a gentle stream of nitrogen. After drying, the remainder of the procedure was the same as for the isoamyl derivatives.

Gas Chromatography/Mass Spectroscopy Analysis

The GC/MS conditions utilized for amino analyses were as follows:

Mass spectrometer sensitivity	10^{-7} ams/volt
Electron energy	70 eV
Injector temperature	240°C
Column temperature	
Initial	50°C
Final	280°C
Temperature programming rate	4°/min
Carrier gas	Helium
Gas flow	20 ml/min

Identification of the amino acids was confirmed using known fragmentation mechanisms (138). Quantification was accomplished by comparing the samples against a standard mixture of 20 amino acids of known weight. Any unresolved peaks were quantified by limited mass search to determine peak areas. The limited mass search procedure allows one to obtain a chromatogram representing the response for a particular mass fragment.

Reaction of Some Amino Acids with Hypochlorous Acid

Serine, threonine, alanine, valine, tyrosine, and tryptophan were chosen to test the reaction of amino acids and HOCl. The HOCl solutions were prepared by bubbling chlorine gas into organic free water and checking the HOCl concentration by the OTA method (140). One ml of a 20 μmole/ml solution of each of the above amino acids except tryptophan was placed in separate 5 ml reaction vials and adjusted to pH 1 to 2 with 6N HCl. One ml of 2000 mg/l (28 μmoles Cl_2/ml) of aqueous chlorine was added to each vial. The vials were capped, shaken, and allowed to stand for 30 minutes at room temperature. Controls were also run on each of the amino acids. The control consisted of 1 ml of the amino acid solution and 1 ml of the water used to make the HOCl solution. After 30 minutes, each vial was extracted by adding one ml of ether, capping and shaking vigorously by hand for one minute. The ether extract was analyzed by GC/MS for identification of any ether soluble reaction products with the only change in GC/MS conditions being the initial temperature of the column (30°C). Three microliters of the aqueous layer were also run by GC/MS using direct aqueous injection and the conditions previously listed. The remainder of the aqueous solution was dried under a stream of nitrogen and the volatile n-propyl derivatives of the amino acids were prepared and analyzed by GC/MS.

A solution which contained 76.8 mg (376 μmoles) of tryptophan was reacted at ambient pH with 3.3 ml of a HOCl solution which contained 13.3 mg (188 μmoles) of chlorine for 30 minutes at room temperature. The reaction mixture was then extracted with ether, using the same procedure as above. The ether extract which contained a red colored product was analyzed by GC/MS under the same conditions as previous ether extracts. The ether material did not yield any gas chromatograph peaks. The amino acid derivatization procedure was carried out on the red product in ether and the derivative mixture analyzed by GC/MS. The aqueous portion of the reaction mixture was dried under a stream of nitrogen and derivatized using the procedure described above. In both cases N(O)-heptafluorobutyryl n-propyl esters were prepared (see below).

Analysis of Sewage Sample

Sewage product samples from four cities were analyzed for amino acids. The cation and ligand exchange procedure was used to concentrate the amino acids as described in Figure 19. Derivatives were prepared using the HFBA acylation and esterification procedures as previously described. City A sample consisted of 100 ml of raw sewage. The sample from City B was 25 liters of anaerobic digestor supernate which had been "superchlorinated" at the City plant. This sample was quenched on site with sodium sulfite to remove residual chlorine. The sample from City C consisted of combined primary and secondary sludges which were also "superchlorinated" at the City's plant. Two liters of this sample were quenched with sodium sulfite after two hours. Another two liters of the sample from City C was quenched with sodium sulfite after four days. Samples from City D were one liter of "superchlorinated" septage which was chlorinated at the City's plant and one liter of raw septage. Both samples were quenched on site with sodium sulfite. Any particulate matter was removed from the samples by filtering through 50 cm dia. Whatman prefolded filter paper or centrifugation and filtering.

RESULTS AND DISCUSSION

Analysis of Amino Acid Standards

Analysis of the amino acids was first attempted using the procedure described above in which the *isoamyl* esters were prepared using tri*iso*amoxymethane, followed by acylation with HFBA. It was found that histidine and tryptophan did not derivatize by this method, and that arginine gave very low yields. It was decided then to use the method of Moss and Lambert (134); that is, esterification with n-propyl alcohol which was 8M in HCl, followed by acylation with HFBA.

Since cysteine is partially oxidized to cystine (134, 136) by this procedure, the quantification of cysteine or cystine must be taken with reservation. Moreover, the analysis of histidine showed reproducibility problems (133) since the compound was partially destroyed during derivatization.

Analysis of the derivatized amino acids can be done by GC alone; however, interfering peaks (141) or poor resolution severely limit this method. Since the GC/MS system can be used to separate unresolved or interfering peaks by the limited mass search procedure and identification of the amino acids can be made using known fragmentation mechanisms (138), it was the system selected for this work. Fragmentation patterns for aromatic and aliphatic amino acids are shown in Figures 20 and 21 (138). A total ion chromatogram of the 20 amino acids derivatized by this method is shown in Figure 22.

Minimum Detectable Limits of the N(O)-Heptafluorobutyryl Alkyl Esters by GC/MS

Since the minimum detectable limit (MDL) of the GC/MS system for the derivatized amino acids was not known, a study was performed to determine this limit. These studies were performed using the HFB-*n*-propyl and HFB-*isoamyl* esters of the amino acids. A sensitivity setting of 10^{-7} amps/volt on the GC/MS system was used since the 10^{-8} amps/volt setting produced too much noise. Constant volumes of solutions of the derivatized amino acids at different concentrations were run on the GC/MS system and a signal to noise ratio of 2 was used as the criterion for minimum detectable limit. That is, MDL was determined as the amount of the derivative necessary to give a S/N level of 2. A single run of the HFB-*isoamyl* esters was made as a comparison, and two microliters of a 0.5 μmole/ml solution of the amino acids were injected in all cases. As can be seen in Table 11, the minimum detectable limit of histidine is high relative to the remainder of the amino acids. Histidine either does not derivatize completely or the imidozole ring may react with the column packing material (131, 132).

Reaction of Some Amino Acids with Hypochlorous Acid (Chlorine Water)

Serine, threonine, alanine, valine, leucine, phenylalanine, tyrosine, and tryptophan were chosen to test the reaction of amino acids and HOCl since they are typical of the aromatic, aliphatic and hydroxyamino acids. The reaction and GC/MS conditions were as described earlier in this section. The neutral reaction products obtained from ether extraction and those obtained by the HFB-*n*-C_3H_7 derivatization procedure are listed in Table 12.

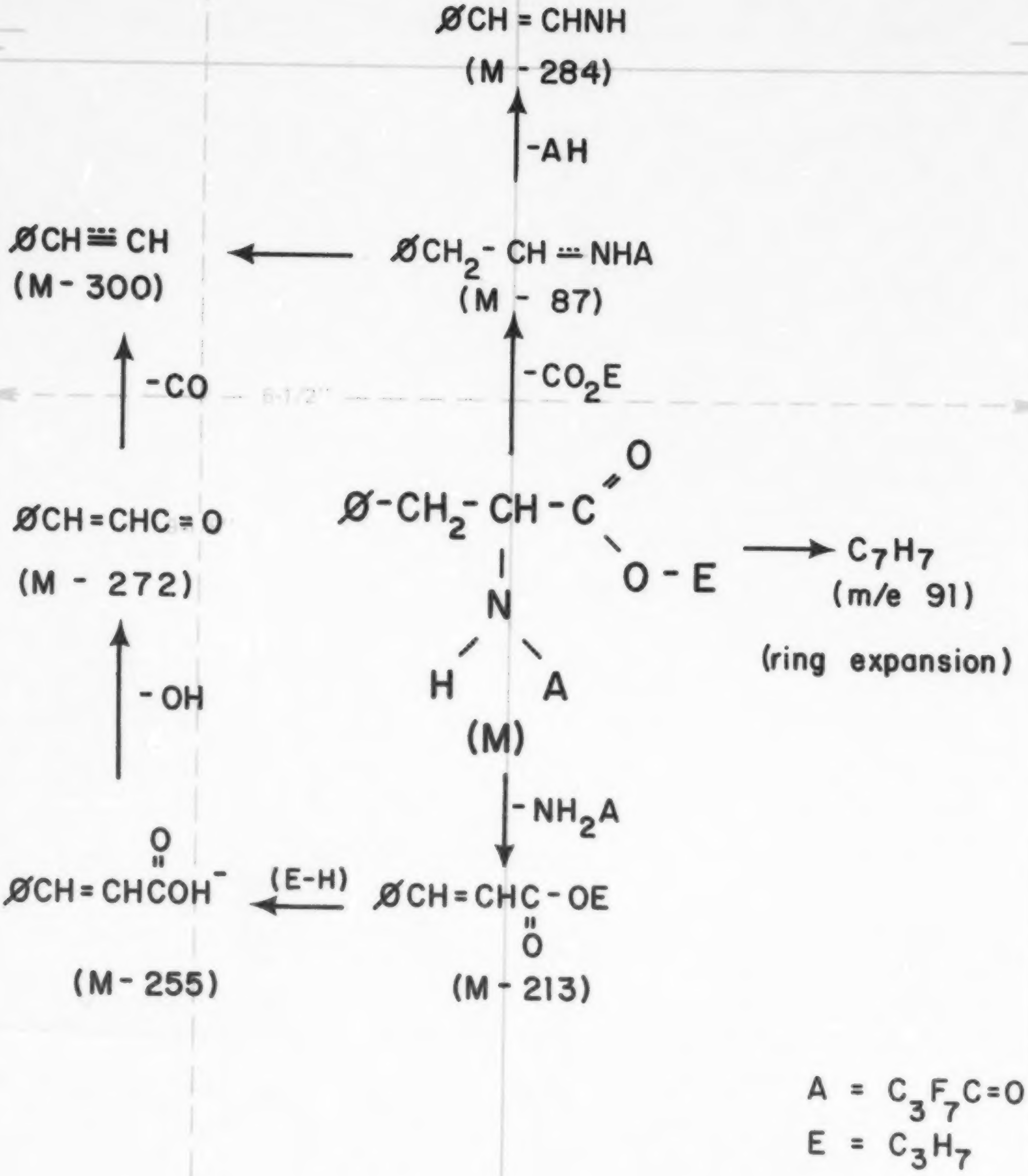
ØCH = CHNH
(M - 284)
-AH
ØCH ≕ CH
(M - 300)
ØCH₂ - CH ≕ NHA
(M - 87)
-CO
-CO₂E
ØCH = CHC = O
(M - 272)
Ø-CH₂- CH - C(=O) - O - E
N
H A
(M)
C₇H₇
(m/e 91)
(ring expansion)
-OH
-NH₂A
ØCH = CHC(=O)OH⁻
(E-H)
ØCH = CHC(=O) - OE
(M - 255)
(M - 213)
A = C₃F₇C=O
E = C₃H₇

Figure 20. Fragmentation of aromatic amino acids (N(O)-heptafluorobutyryl propyl esters). All species have +1 formal charge.

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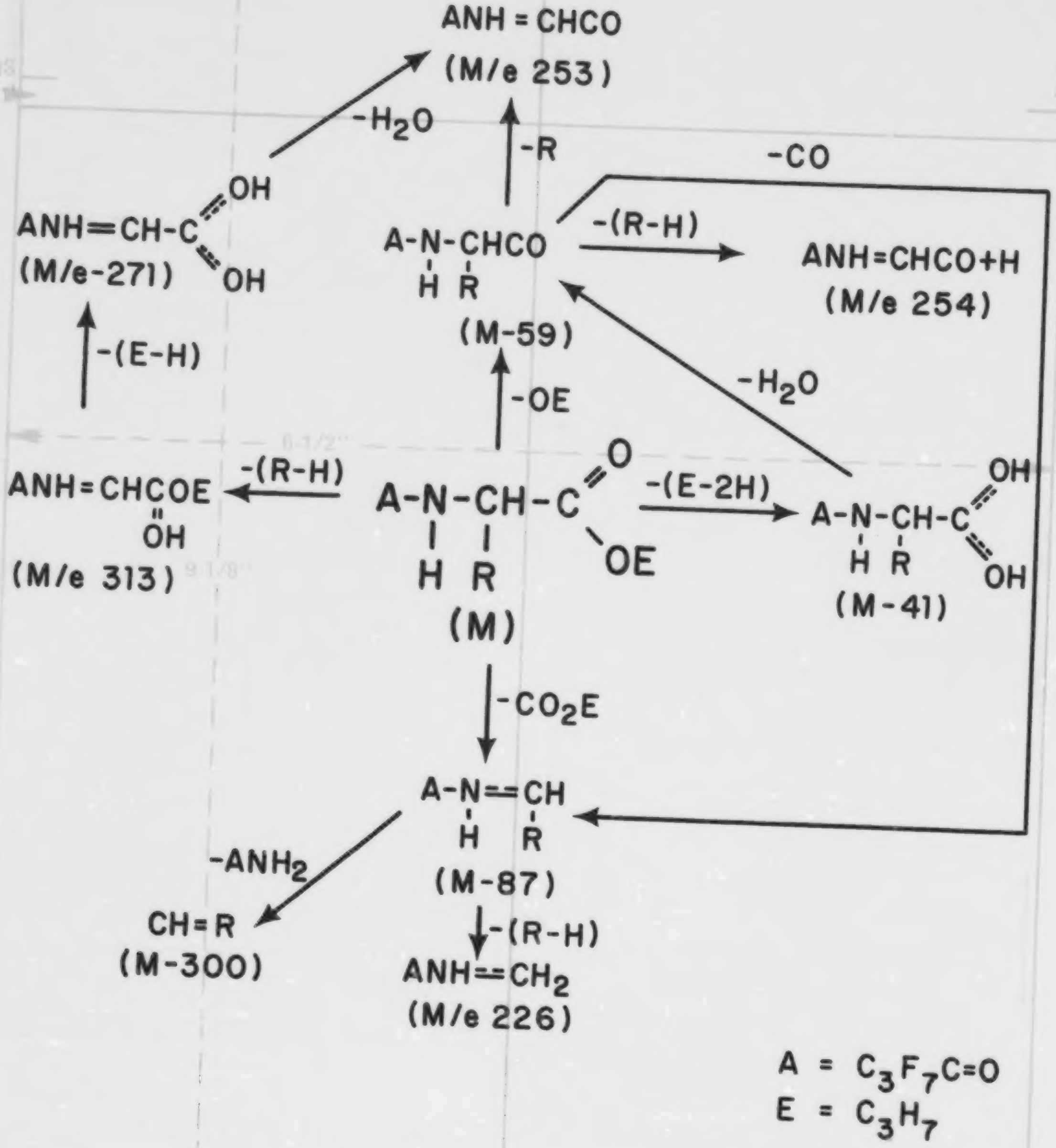
ANH = CHCO
(M/e 253)
-H2O
-R
-CO
ANH=CH-C OH OH
(M/e-271)
A-N-CHCO H R
(M-59)
-(R-H)
ANH=CHCO+H
(M/e 254)
-(E-H)
-H2O
-OE
ANH=CHCOE OH
(M/e 313)
-(R-H)
A-N-CH-C O OE H R
(M)
-(E-2H)
A-N-CH-C OH OH H R
(M-41)
-CO2E
A-N=CH H R
(M-87)
-ANH2
CH=R
(M-300)
-(R-H)
ANH=CH2
(M/e 226)
A = C3F7C=O
E = C3H7

Figure 21. Fragmentation of aliphatic amino acids (N(O)-heptafluorobutyryl, n-propyl esters). All species have +1 formal charge.

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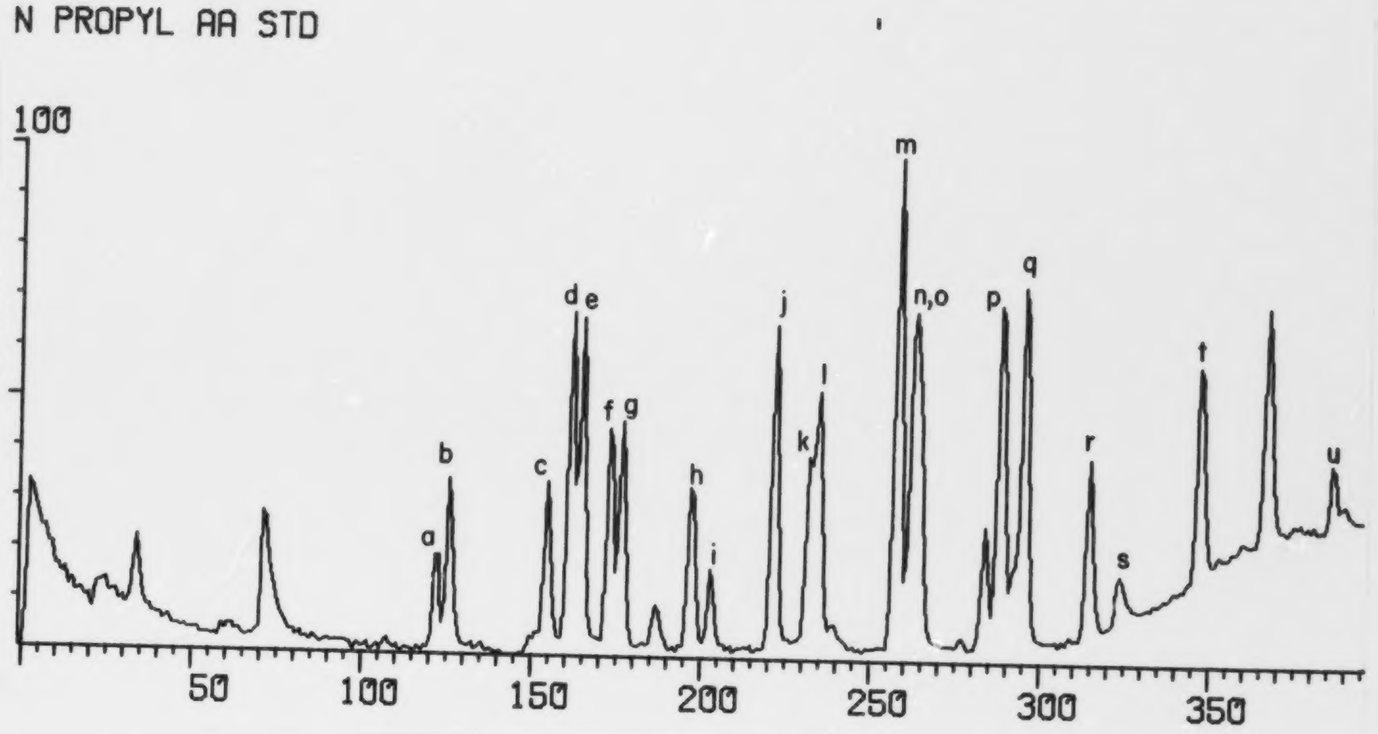

Figure 22. Reconstructed GC/MS chromatogram of amino acid standard: a-alanine; b-glycine; c-valine; d-threonine; e-serine; f-leucine; g-isoleucine; h-proline; i-cysteine; j-methionine; k-aspartic acid; l-unknown impurity; m-phenylalanine; n,o-glutamic acid, ornithine; p-lysine; q-tyrosine; r-arginine; s-histidine; t-tryptophane; u-phthalate.

TABLE 11. MINIMUM DETECTABLE LIMIT OF N(O)-HEPTAFLUOROBUTYRYL ALKYL ESTERS OF AMINO ACIDS[a]

Amino Acid	MINIMUM DETECTABLE LIMIT (ng)				
	n-Propyl Ester				Isoamyl Ester
	Run 1	Run 2	Run 3	Avg.	
Alanine	3.6	4.4	3.9	4.0±0.3	10.8
Glycine	2.4	2.8	2.6	2.6±0.2	13.5
Valine	4.1	3.2	2.8	3.4±0.5	10.8
Threonine	1.2	1.6	1.5	1.4±0.2	2.6
Serine	1.1	1.3	1.2	1.2±0.1	4.0
Leucine	4.0	3.8	3.7	3.8±0.1	3.1
Isoleucine	3.9	3.6	3.5	3.7±0.2	5.6
Proline	4.0	4.4	4.6	4.3±0.3	2.1
Cysteine	3.2	3.3	3.9	3.5±0.3	18.9
Tryptophan[b]	17.0	19.5	20.0	18.8±1.2	--
Hydroxyproline	4.1	3.7	3.5	3.8±0.3	2.8
Methionine	6.9	6.6	6.9	6.9±0.2	6.3
Histidine[c]	151	167	182	167±10	--
Phenylalanine	1.4	1.6	1.5	1.5±0.1	8.9
Ornithine	3.7	3.9	3.8	3.8±0.1	7.9
Lysine	4.4	4.0	3.9	4.1±0.2	8.5
Tyrosine	4.5	5.4	5.2	5.0±0.4	7.7
Aspartic Acid	4.4	3.8	3.7	4.0±0.3	6.5
Arginine[d]	7.0	7.3	8.7	7.7±0.7	--
Glutamic Acid	5.8	6.3	6.5	6.2±0.5	9.5

[a] GC/MS conditions as indicated in text

[b] Derivatization as *iso*amyl ester does not recover tryptophan

[c] Derivatization as *iso*amyl ester does not recover histidine

[d] Arginine not in standard for *iso*amyl ester

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The reaction products of HOCl and amino acids in general were a confirmation of previous work (96-107). Major exceptions are the chlorotyrosine and the dichlorotyrosine which had not been confirmed before in reactions of HOCl and tyrosine. The oxindole derivatives of tryptophan were also very unusual since these compounds have not been reported by other workers and the fact that further carbon-chlorine bonding was shown.

The mass spectra of N(O)-HFB-n-propyl esters of mono and dichlorotyrosine are shown in Figures 23 and 24 respectively. Figures 20 and 21 rationalize the major peaks in terms of a plausible fragmentation pattern. The spectra in Figures 23 and 24 actually were obtained on compounds found in the sample from C after four days of "superchlorination" but are identical to those of products found in the laboratory chlorination of tyrosine. Peaks at m/e of 436, 394, 377, 349, and 337 for chlorotyrosine and m/e of 470, 428, 411, 383, and 372 for dichlorotyrosine indicate that chlorination is on the aromatic ring, presumably at the 3 and 5 positions, but the precise position cannot be ascertained from mass spectrometry alone.

Cation and Ligand Exchange Recovery Studies

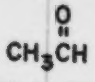
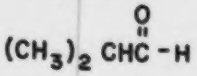
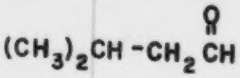
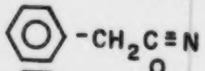
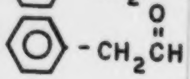
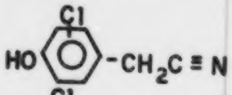
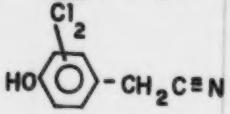
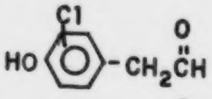
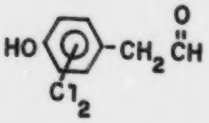
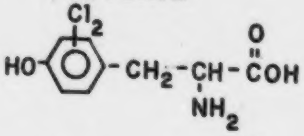
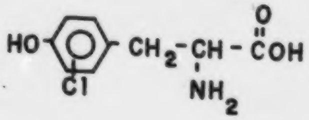
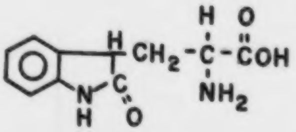
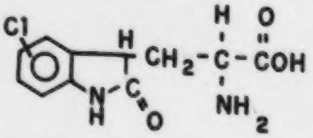
To determine the recovery efficiency of 20 amino acids from the cation and ligand exchange procedure described earlier in this section, 50 μliters of a standard amino acid solution at the 10 μmoles/ml concentration were spiked into two liters of water and passed through the exchange procedure. The final effluent was dried and derivatized as the N(O)-heptafluorobutyryl n-propyl esters as before. Fifty μmoles of the same mixture were derivatized along with the recovery samples as a control. The recovery efficiencies from the runs listed in Table 13 vary from 101.9% for glycine to 69.2% for histidine.

Comparison of GC/MS Method for Amino Acid Analysis with a Beckmann Model 120-C Amino Acid Analyzer

To compare the two methods, using a wastewater matrix, two liters of the final effluent from a city sewage plant were obtained. The final effluent was quenched with excess sodium sulfite to remove residual chlorine and then filtered. The two liter sample was spiked with a standard mixture of 20 amino acids at the 5 nmoles/ml level. Two one ml samples of this solution were run on the Beckmann Model 120-C amino acid analyzer and the remainder of the sample was split and analyzed by the GC/MS procedure. The cation and ligand exchange for clean up and concentration of the amino acids, and the n-propyl esterification and HFB acylation procedures described earlier in this section were used.

In general, both methods gave good results, as can be seen in Table 14. The amino acid analyzer gave a low value

TABLE 12 REACTION PRODUCTS IDENTIFIED FROM THE REACTION OF AMINO ACIDS WITH HOCl

AMINO ACID	PRODUCTS IN ETHER EXTRACT	PRODUCTS IN AQUEOUS SOLUTION
SERINE	NONE	SERINE
ALANINE	$CH_3C\equiv N$  $CH_3\overset{O}{\overset{\|}{C}}H$	ALANINE
VALINE	$(CH_3)_2CHC\equiv N$  $(CH_3)_2\,CH\overset{O}{\overset{\|}{C}}-H$	VALINE
THREONINE	NONE	THREONINE
LEUCINE	$(CH_3)_2\;CHCH_2C\equiv N$  $(CH_3)_2CH-CH_2\overset{O}{\overset{\|}{C}}H$	LEUCINE
PHENYLALANINE	 	PHENYLALANINE
TYROSINE	   	TYROSINE  
TRYPTOPHAN	NONE	 

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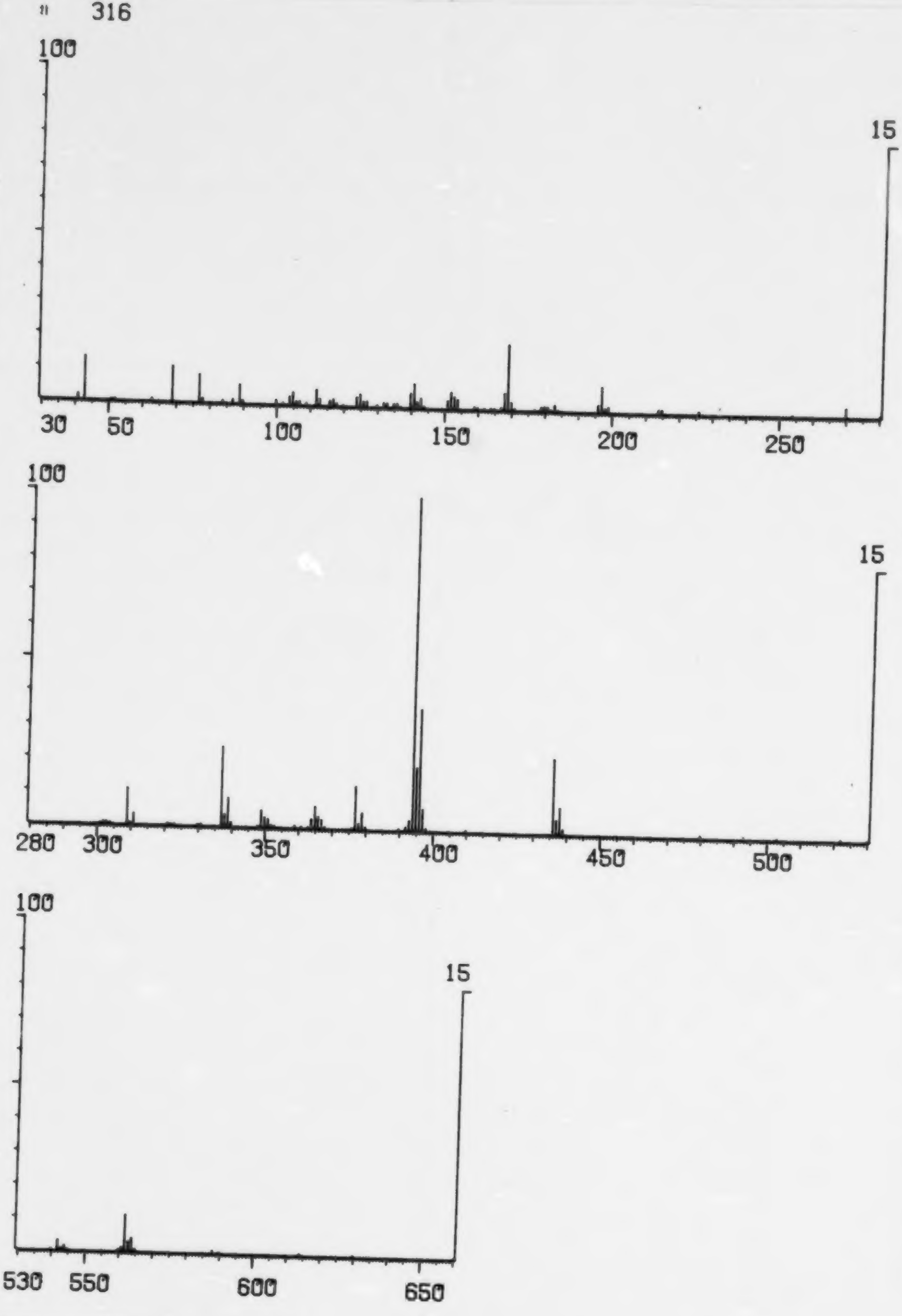

Figure 23. Mass spectrum of chlorotyrosine.

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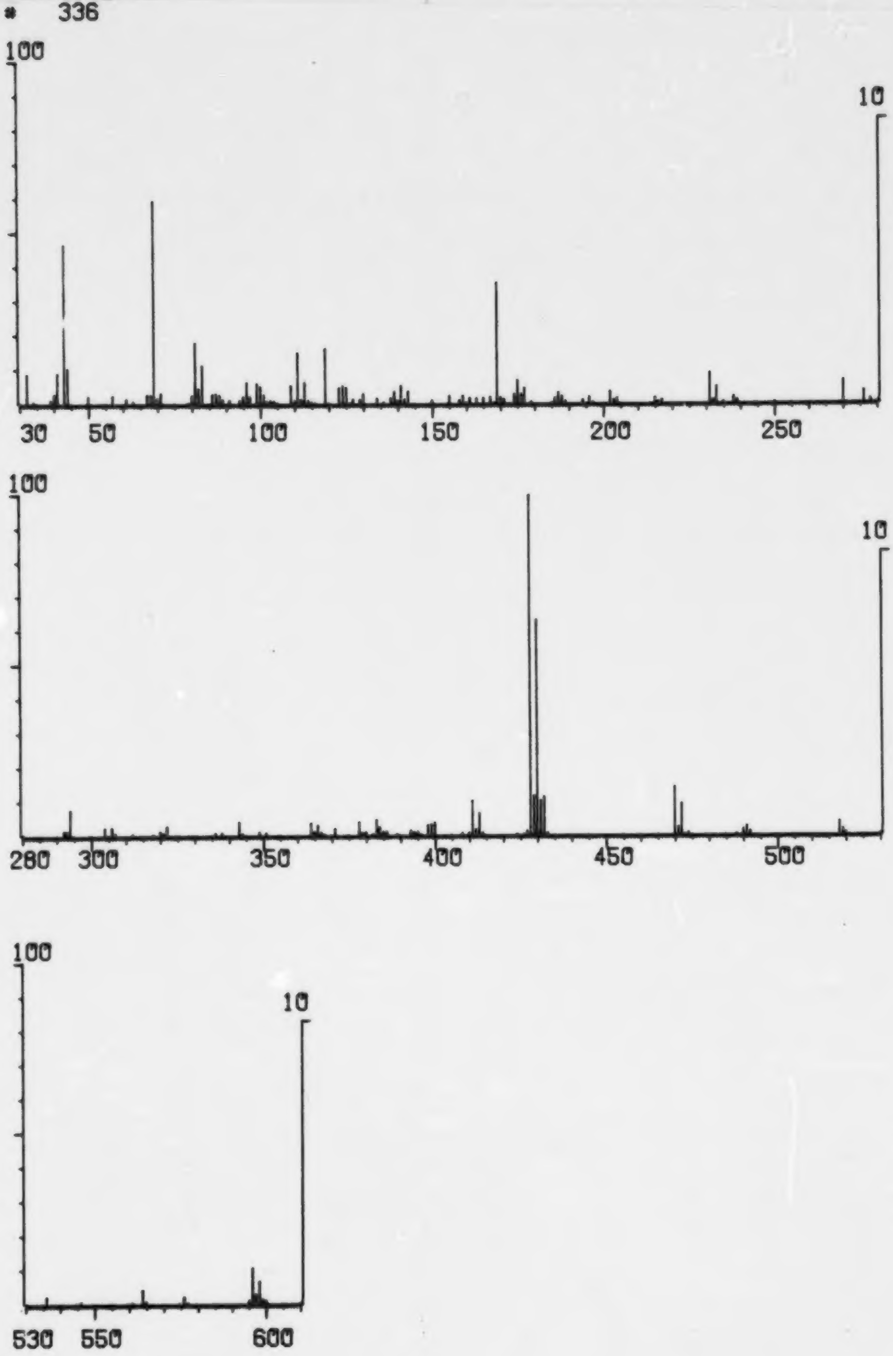

Figure 24. Mass spectrum of dichlorotyrosine.

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TABLE 13. RECOVERY EFFICIENCIES OF 20 AMINO ACIDS BY THE COMBINATION OF THE CATION AND LIGAND EXCHANGE RESINS

Amino Acid	Average[a] Per cent recovery
Alanine	88.9 ± 0.2
Glycine	101 ± 2
Valine	95 ± 1
Threonine	93.9 ± 1
Serine	93.4 ± 0.4
Leucine	94.7 ± 0.3
Isoleucine	91.2 ± 2
Proline	91.7 ± 1
Cysteine	91.8 ± 2
Hydroxyproline	95.1 ± 2
Methionine	94.6 ± 2
Aspartic Acid	99.4 ± 6
Phenylalanine	93.5 ± 2
Glutamic Acid	82.8 ± 3
Ornithine	90.6 ± 1
Lysine	81.3 ± 2
Tyrosine	89.3 ± 1
Arginine	80.6 ± 2
Histidine	69.2 ± 2
Tryptophan	82.4 ± 3

[a] Based on duplicate runs

for cysteine, while the GC/MS system obtained a value close to theoretical; also, the amino acid analyzer would not elute hydroxyproline. The GC/MS value for histidine was relatively large and is likely related to the problems with histidine mentioned earlier (131,133). Overall, the comparison was very good with both methods having about a 90% efficiency. The average difference between the two methods was 12.8% with a maximum and minimum of 48.8% and 1.40% respectively. In general, precision was superior by the GC/MS method.

Analysis of Municipal Waste Samples for Amino Acids

Four types of municipal waste samples from four cities were analyzed for free amino acids. The cation and ligand exchange procedure was used for cleanup and concentration as described earlier in this section. Derivatives were prepared using the HFBA acylation and the *n*-propyl esterification procedure. The total ion chromatogram of the derivatized amino acid extract of the superchlorinated septage is shown in Figure 25.

Table 15 shows the concentrations of amino acids found in these samples. It is interesting to note the levels found in raw sewage of City A differ in a few cases from those reported in the literature. Presumably, this is due to the nature of the plant influent. As expected, amino acid levels are higher in septage; however, it can be seen that "superchlorination" is an effective method for destroying the amino acids. Since chlorine doses used in this method are typically in the 0.2-0.4% range, under these conditions one would expect extensive oxidation of organic materials to take place. In addition, one would expect to observe considerable formation of chlorinated organic products, which has been confirmed by Glaze and Henderson (75). Parallel work on the samples listed in Table 15 by other members of the North Texas State University research team has shown that numerous chloro-organics are present in superchlorinated samples from Cities B, C, and D (Cf. section 5 of this report).

Table 15 shows that these samples also contain chlorotyrosine, and in one case dichlorotyrosine. This observation represents the first confirmation of a chlorinated amino acid in a wastewater product. The occurrence of chlorinated amino acids in chlorinated wastes was predicted quite earlier by persons such as Robert Dean at the United States Environmental Protection Agency, Cincinnati, Ohio, in a personal communication to William H. Glaze.

The significance of these chlorinated products in wastewater plant products cannot be stated at this time. The fate of the compounds in the receiving environment undoubtedly will depend on the choice of disposal method, as well as the

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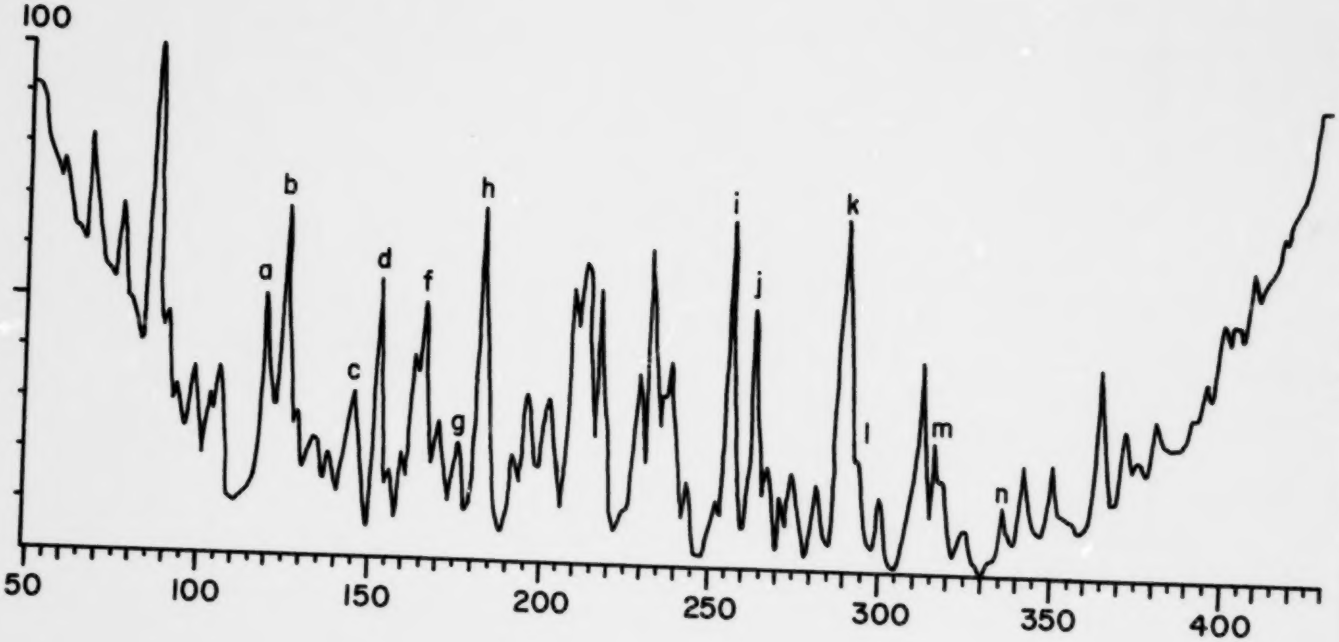

Figure 25. Reconstructed GC/MS chromatogram of amino acid extract from superchlorinated septage extract. a-alanine; b-glycine; c-valine; d-threonine; f-serine; g-leucine; h-isoleucine; i-phenylalanine; j-glutamic acid; k-lysine; l-tyrosine; m-chlorotyrosine; n-dichlorotyrosine.

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TABLE 14. COMPARISON OF GC/MS ANALYSIS OF DERIVATIZED AMINO ACIDS WITH ANALYSIS BY A BECKMANN AMINO ACID ANALYZER USING A WASTEWATER MATRIX.

Amino Acid	Spiked Concentration (µm/l)	Amino Acid Analyzer Concentration Found (µm/l)			GC/MS Concentration Found (µm/l)		
		1	2	Avg.	1	2	Avg.
Alanine	5.08	5.29	5.29	5.29+0.01	4.98	4.83	4.91+0.08
Glycine	5.00	5.02	4.58	4.80±0.22	4.51	4.33	4.42±0.09
Valine	5.00	4.20	5.01	4.61±0.41	4.59	4.89	4.74±0.15
Threonine	5.18	5.04	4.70	4.87±0.17	4.73	4.66	4.70±0.03
Serine	5.00	4.43	4.51	4.47±0.04	4.74	4.58	4.66±0.08
Leucine	5.00	4.43	4.38	4.41±0.02	4.57	4.54	4.56±0.01
Isoleucine	5.19	5.12	4.81	4.97±0.15	5.07	5.02	5.04±0.03
Proline	5.00	5.18	5.11	5.15±0.03	4.72	4.70	4.71±0.01
Cysteine	5.00	2.70	2.13	2.42±0.28	5.24	5.38	5.31±0.07
Hydroxyproline[a]	5.00	-	-		4.64	4.56	4.60±0.04
Methionine	5.00	4.11	3.67	3.89+0.22	4.57	4.51	4.54±0.03
Aspartic Acid	5.00	5.29	5.16	5.23±0.06	4.45	4.38	4.41±0.04
Phenylalanine	5.00	4.52	4.25	4.39±0.13	4.64	4.54	4.59±0.05
Glutamic Acid	5.00	5.55	5.38	5.47±0.08	4.95	5.28	5.12±0.16
Ornithine[b]	5.00	-	-		4.52	4.58	4.55±0.03
Lysine	5.00	5.19	4.88	5.04+0.15	5.02	4.91	4.96±0.06
Tyrosine	5.00	4.41	3.89	4.15±0.26	4.76	4.65	4.71±0.05
Arginine	5.00	4.94	4.72	4.83±0.11	4.80	4.68	4.74±0.06
Histidine	5.11	4.47	4.32	4.40±0.07	6.84	7.65	7.24±0.41
Tryptophan	5.00	5.06	5.43	5.25±0.18	4.67	4.55	4.61±0.06

[a] Hydroxyproline not eluted from amino acid analyzer column.

[b] Ornithine not in standard used on amino acid analyzer.

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TABLE 15. AMINO ACIDS PRESENT IN MUNICIPAL WASTES, µg/l

		CITY A	CITY B	CITY C COMBINED SLUDGE		CITY D SEPTAGE	
	Typical[a] Domestic Sewage	Raw Sewage	Super-Chlorinated Anaerobic Digestor Supernatant	2-hour Super Chlorination	4-day Super Chlorination	Raw	Super-chlorinated
Alanine	20 - 90	250	4.0	3.1	3.2	1,220	8.3
Glycine	20 - 130	44	24.0	8.9	5.1	1,410	5.6
Valine	50 - 240	200	0.9	11.1	3.2	780	4.0
Threonine	10 - 180	120	3.0	0.9	tr	450	0.9
Serine	20 - 130	37	36.9	2.4	1.2	400	1.2
Leucine	60 - 280	380	tr	7.9	tr	960	2.7
Isoleucine		130	8.0	7.1	5.8	580	1.3
Proline	0	42	3.0	<0.11	<0.04	100	<0.07
Cysteine		<7	<0.07	<0.09	<0.03	60	<0.05
Hydroxyproline		52	<0.08	<0.10	<0.04	100	<0.06
Methionine	50 - 240	19	<0.14	<0.17	<0.07	110	<0.10
Aspartic Acid	20 - 130	82	<0.08	<0.10	<0.04	130	<0.06
Phenylalanine	20 - 330	88	5.0	10.5	3.6	300	2.1
Glutamic Acid	10 - 180	52	3.0	15.1	4.5	150	0.4
Lysine	tr	110	5.0	3.5	3.6	130	1.9
Arginine	tr	<15	<0.15	<0.19	<0.07	750	<0.12
Histidine	tr	130	<3.3	<4.2	<1.6	370	<2.5
Tyrosine	60 - 90	150	3.0	1.4	1.1	450	0.4
Chlorotyrosine		<10[b]	3.0[b]	1.0[b]	1.3[b]	<5[b]	0.2
Dichloro-tyrosine		<10[b]	<0.10[b]	<0.12[b]	0.5[b]	<5[b]	<0.8

[a] Reference 32 < signifies approximate detection limit for the respective amino acid.

[b] Based on detector response to tyrosine

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stability of the particular compound. In the case of "superchlorinated" sludge or septage products, there may be some concern for the leaching of compounds into receiving streams of ground water if sludge beds are not adequately sealed. In any case, these data and the results of earlier works point out the need for caution in the disposal of "superchlorinated" waste products to avoid possible contamination of the environment.

SECTION 7

THE ANALYSIS OF NON-VOLATILE ORGANIC COMPOUNDS IN WATER AND WASTEWATER AFTER CHLORINATION

INTRODUCTION

This section focuses on the non-volatile organic constituents of natural waters and municipal wastewaters before and after chlorination. Of particular interest is the development of high performance liquid chromatography (HPLC) approaches to the study of these systems. New information is presented on the molecular size dispersion of non-volatile organic substances in water and their role in the formation of trihalomethanes and other chlorinated by-products.

BACKGROUND

Section 4 summarizes current knowledge on the structure and composition of naturally occurring organic substances in water. As noted there, soluble aquatic organic matter is largely of the "fulvic acid" type, that is, it is soluble in both mineral acid and base (Figure 3, Section 4). There is still controversey over the chemical structure of fulvic acid; indeed, it is almost certain that a single "structure" cannot be written for the material. The structures proposed in Figures 4 and 5 in Section 4 represent composite structures based principally on chemical degradation products, and the persons responsible for the structures are among the first to point out that the actual composition of a natural organic matrix is much more complicated.

The approach taken in this study was to concentrate on the development of new HPLC-based methods to separate the natural matrix into its components. This approach was based mostly on the intuitive judgement that more could be learned about natural organics and their by-products if the systems were first simplified by the use of modern separation methods. High performance liquid chromatography offers several modes for the study of aqueous organic compounds (145). While the study reported here is by no means complete, significant new data is reported on the fractionation of aquatic organic matrices, and on the effects of disinfection agents on these fractions.

EXPERIMENTAL PROCEDURES

Purified Water

The water used to prepare reagents and for TOX and LLE blanks came from one of two systems. The first system, which produced water referred to as "D" water, was described in the work of Glaze and others (71). This process began with ozonated, high-purity-bottled water supplied by Ahlfingers Water Corporation. This water was further purified by passage through a 1.5 cm i.d. glass column that was filled with 50 cm Filtrasorb F-400 activated carbon (12/40 mesh), followed by 25 cm of XAD-2 (20/50 mesh), and finally a Millipore glass-filter disc. This purified water was analyzed for total organic carbon (TOC) and was found to contain less than 200 ppb. The total organic halogen (TOX) value for this water by the GAC method (146) was found to be 15 to 20 ppb. No detectable THMs were found.

The second type of water was supplied via a commercial water-treatment apparatus from Millipore. This system produced so-called "Q" water; it consists of a tap-water feed to a series of cartridges consisting of Rogard prefilter, reverse osmosis, granular activated carbon, and two ion-exchange cartridges. Typically, this water gave the following measured values: TOC--300-500 ppb; TOX--5-10 ppb; and no detectable THMs.

High Performance Liquid Chromatography

Two instruments were utilized. The first is a Waters ALC-201 with 6000A pump and built-in refractive index detector. The second is a Micromeritics 7000B. Three detectors were used with both instruments: a Tracor 970-A scanning UV detector; a Schoeffel FS970 fluorometer; and a modified Coulson electrolytic conductivity detector originally designed as a GC detector. Off-line detection of halogen content of HPLC effluents was accomplished by manual collection of fractions, followed by pyrolysis/microcoulometry with a Dohrmann MCTS-20 system.

HPLC exclusion (molecular size) separations were carried out with two columns containing Partisil 10 (Whatman, 11 μ particle size, 60 A pore size) deactivated with bonded glycerylpropylsilane according to the procedure of Regnier and Noel (147). The characteristics of the two columns are shown in Table 16. Columns were packed by the upward slurry technique of Bristow _et al._ (148) and coated with Carbowax 20M in most cases. Carbon filtered "Q" or "D" water was used as carrier solvent, but 2% isopropanol/water also was used in earlier work. The working range of the column was determined with a combination of proteins and sodium polystyrene sulfonates of known

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TABLE 16. CHARACTERISTICS OF PARTISIL 10/GLYCOPHASE HPLC COLUMNS

	Analytical	Preparative
Length (cm)	25	25
ID (mm)	4.6	9.4
Number of Theoretical Plates N	2500	3500
HETP (mm)	0.1	0.07
Linear Velocity (cm/sec)	0.185	0.097
Void Volume V_o (mL)	2.25	3.1
Permeation Volume V_t (mL)	4.9	7.4
Interstitial Volume V_i (mL)	2.65	4.3

Packing Material: Partisil 10 (Whatman 11 μ particle size, 60 Å pore size) deactivated with Glycerol-propyl silane and treated with Carbowax 20M.

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molecular weight. As shown in Figure 26, the exclusion limit of the column is 45,000 (k'=0), but the practical range is probably from 30,000 - 1,500.

Weak anion exchange chromatography was carried out using a 98 cm X 1.5 mm I.D. column containing AL Pellionex WAX, a weakly basic anion exchanger (Whatman). Gradient elution was used beginning with an acetic acid solution (pH3) and ending with a triethylamine-acetic acid buffer (pH6.5). Both linear and concave N^3 gradients were utilized.

Reverse phase HPLC used a preparative column (25 cm X 9.4 mm ID) and an analytical column (25 cm X 3 mm ID). The former was prepacked with Whatman Magnum-9 Partisil-10 ODS-2 and the latter was slurry packed in the laboratory with Partisil-10 ODS-2. A linear gradient was used for elution beginning with 5% methanol in acetic acid (pH 3) and ending with 50% methanol in water.

Water Samples

Secondary treated wastewater from the city of Denton, Texas was collected before chlorination and after final clarification. General characteristics of the samples are given in Table 17. It is noted that from 67 to 89 percent of the total organic carbon is passed through a 0.45 μ filter (dissolved organic carbon, DOC).

Lake water was taken from Cross Lake, Louisiana. Some characteristics of this water are shown in Table 18, although it should be noted that variations are expected on a seasonal basis.

Samples were collected in glass bottles which had been rigorously cleaned with detergent, chromic acid cleaning solution, carbon-filtered water, and then dried at 150°C. No attempt was made to collect head space free samples.

Analytical Scheme

The procedure used to isolate and characterize non-volatile organics is shown in Figure 27. The procedure involves freeze-drying of the purged, filtered samples. Redissolution into water, base and acid soluble fractions was attempted initially, but later more emphasis was placed on water and acid soluble fractions. Following redissolution the fractions were examined by various HPLC modes, including characterization of fractions collected by preparative HPLC. Analytical parameters used to characterize the fractions included total organic carbon (TOC) using the Dohrmann DC-54 analyzer, and total organic halogen (TOX) using the Dohrmann GAC method (146) or the XAD-2 method (71). It should be noted that recent work has shown that the latter gives values of TOX substantially lower than the former method, when a chlorinated surface water source is used (149). The reason for this discrepancy presumably is the inability of XAD-2 or

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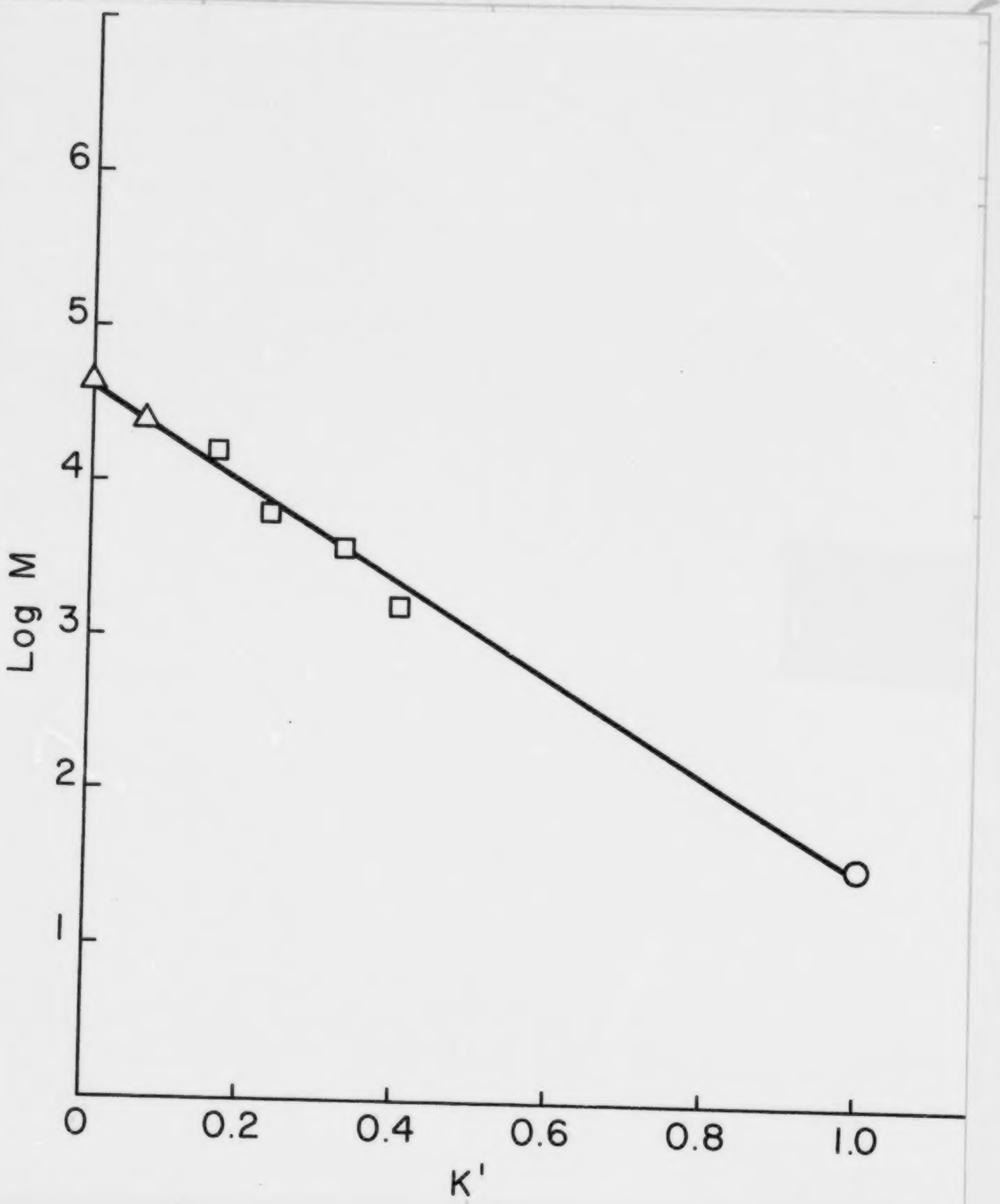

Figure 26. Calibration curve for Partisil 10/glycophase size exclusion columns. △ Proteins (ovalbumin, M=45,000; chymotrypsinogin A,M=25,000). □ Sodium polystyrene sulphonates (M=16,000; 6,500; 4,000, 1,600). ○ Methanol.

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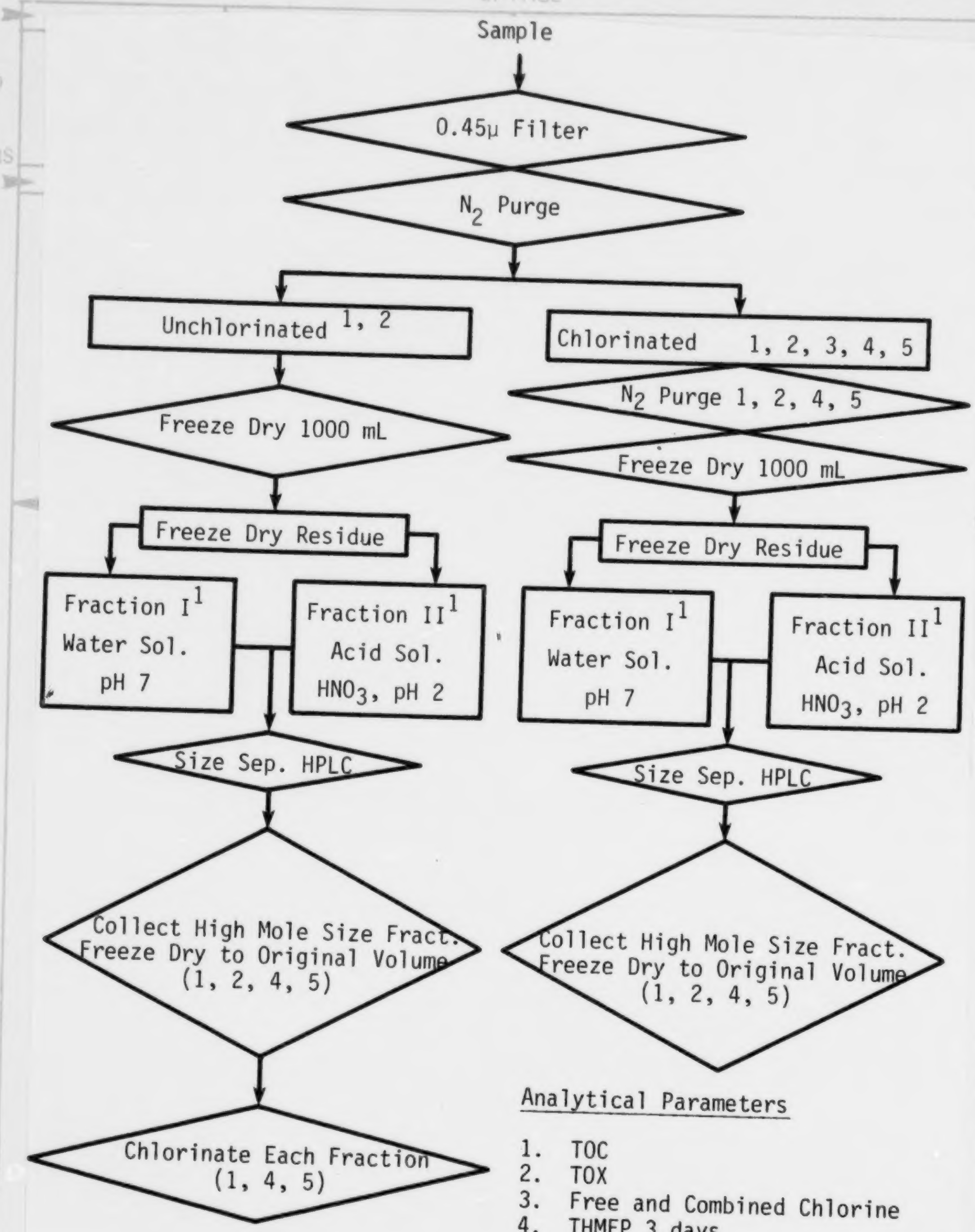
Sample
0.45μ Filter
N2 Purge
Unchlorinated 1, 2
Chlorinated 1, 2, 3, 4, 5
Freeze Dry 1000 mL
N2 Purge 1, 2, 4, 5
Freeze Dry 1000 mL
Freeze Dry Residue
Freeze Dry Residue
Fraction I1 Water Sol. pH 7
Fraction II1 Acid Sol. HNO3, pH 2
Fraction I1 Water Sol. pH 7
Fraction II1 Acid Sol. HNO3, pH 2
Size Sep. HPLC
Size Sep. HPLC
Collect High Mole Size Fract. Freeze Dry to Original Volume (1, 2, 4, 5)
Collect High Mole Size Fract. Freeze Dry to Original Volume (1, 2, 4, 5)
Chlorinate Each Fraction (1, 4, 5)

Analytical Parameters

1. TOC
2. TOX
3. Free and Combined Chlorine
4. THMFP 3 days
5. TOXFP 3 days

Figure 27. Scheme for the study of the effect of chlorination on non-purgeable organics.

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TABLE 17. GENERAL CHEMICAL CHARACTERISTICS OF THE SECONDARY TREATED WASTEWATER

Sample Collection Date	12/7/1978		1/9/1979		1/17/1979	
pH at 25°C	7.6		8.2		7.8	
Specific Cond. (μmho's at 25°C)	877		910		868	
Total TOC (mg/L)	$\overline{X}$ 10.8	S 0.3	$\overline{X}$ 12.7	S 0.4	$\overline{X}$ -	S -
Soluble TOC, before purging (mg/L)	7.2	0.2	11.3	0.1	11.2	0.4
Soluble TOC, after purging (mg/L)	6.8	0.1	11.1	0.1	9.8	0.2
Percent Soluble TOC (before purging)	66.7		89		-	
Percent of Soluble TOC Not Purgeable	94.4		98.2		87.8	

$\overline{X}$ = mean of three injections each 16 ml

S = standard deviation

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TABLE 18. CHARACTERISTICS OF CROSS LAKE WATER (0.45 μ Filtrate)

Color (units)	110
pH @ 25°C	7.5
Ammonia - N (mg/L)	0.2
Organic - N (mg/L)	0.6
Total - P (mg/L)	0.1
COD (mg/L)	35
Soluble TOC (mg/L)	11.0 ± 0.4

TABLE 19. RESIDUAL CHLORINE AT DIFFERENT TREATMENT LEVELS AND CONTACT TIME

Chlorination Level	Residual Chlorine* Free	Combined	mg/L Total
20 mg/L, 1h contact time	1.6	0.7	2.0
30 mg/L, 1h contact time	6.8	2.0	8.8
30 mg/L, 24 hr contact time	4.4	1.8	6.2

Sample: Filtered, purged secondary treated wastewater collected on 12/7/1978

*Analysis by amperometric titration

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XAD-8 resins to trap oxidized natural humics, although they perform satisfactorily on less polar, low molecular weight organics.

Trihalomethane formation potential (THMFP, 150) and TOXFP were determined by THM or TOX analysis after chlorination of samples at a dose of 20 mg/L at pH 6.5 and 25°C. THM analysis was made by the liquid-liquid extraction (LLE) procedure described in Section 8. Residual chlorine was measured by an amperometric procedure (151, Table 19).

RESULTS AND DISCUSSION

HPLC Investigation of a Municipal Drinking Water Source

Fractionation and analysis of the 0.45 μ filtrate of Cross Lake, Louisiana water before and after chlorination followed the schemes shown in Figure 27. Chlorination of the unbuffered lake water at 26°C with a dose of 20 mg/L causes the formation of trihalomethanes (THMs) the yield of which reaches a plateau after three days. At a buffered pH of 6.5 the relative yields of THMs after this period are 81% $CHCl_3$, 16% $CHCl_2Br$ and 3% $CHClBr_2$ (by weight). Expressed as halogen, the yield of total trihalomethanes does not represent the majority of bound halogen in chlorinated natural waters. This observation, which has been extensively documented in this laboratory, is consistent with the works of Sontheimer, Kühn, and co-workers (152) and of Oliver (74).

As expected, the carbon matrix of unchlorinated Cross Lake water is largely a non-volatile fraction, presumably consisting of a mixture of fulvic acid, and other components. After microfiltration and freeze drying, the residue obtained is mostly soluble in purified water (50% of original TOC) and in dilute nitric acid (24% of remaining TOC). A darkly colored solid remains which is partially base soluble and which probably represents humic acids and clay particles which presumably are colloidal in size in the original sample. The water and acid soluble portions are of interest in this work, but it should be noted that the role of fine particulates may be crucial in the transport of micropollutants and in water treatment processes.

After freeze-drying, the water soluble fraction of the Cross Lake sample was analyzed by size exclusion HPLC. Figure 28 shows chromatograms of the water soluble fraction before and after 20 mg/L chlorination for five days (refractive index detector). Apparent average molecular weight has shifted slightly downward upon chlorination as much be expected. Table 20 shows some of the characteristics of five fractions of this water soluble portion collected by preparative HPLC before chlorination. Exclusive chromatograms of the reinjected

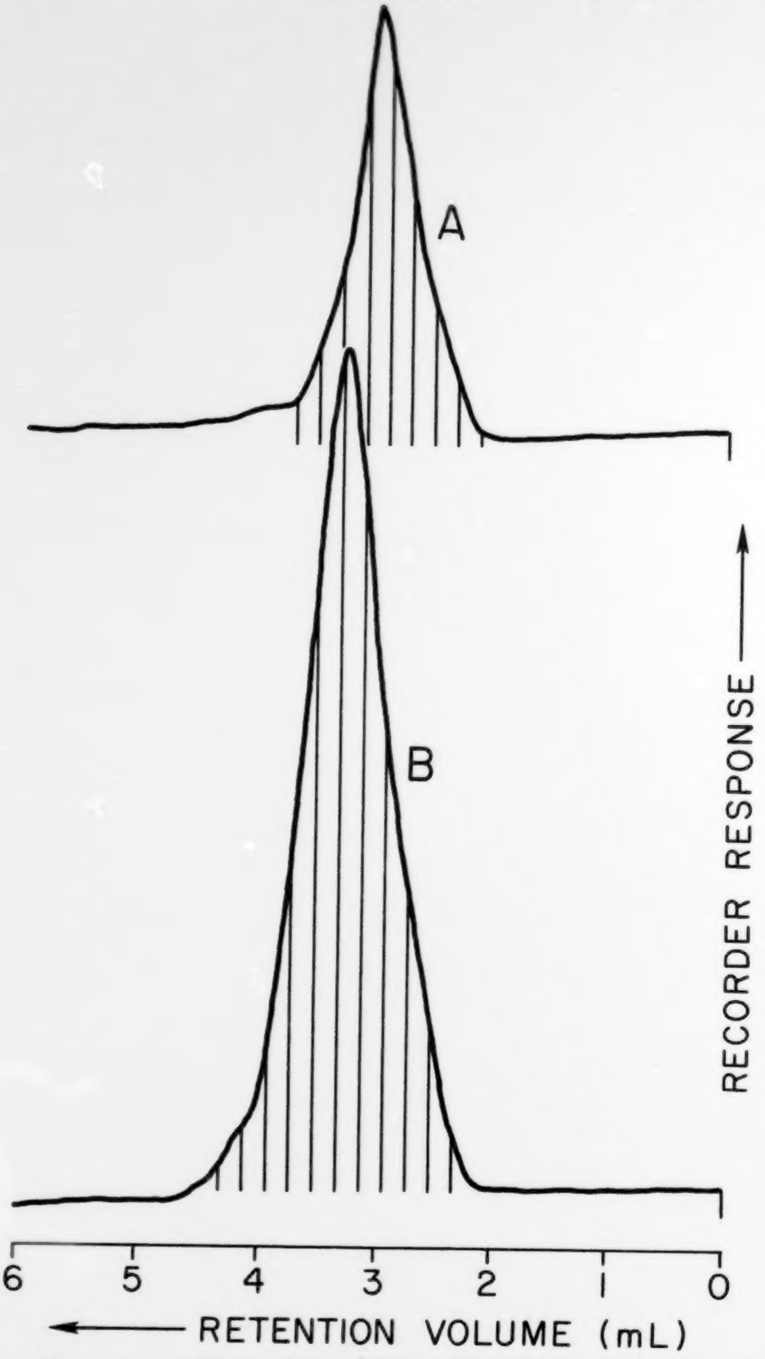

Figure 28. Size exclusion chromatograms of Cross Lake sample, freeze dried water soluble fraction. Top, unchlorinated; bottom, chlorinated at 20 mg/L for five days.

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TABLE 20. CHARACTERISTICS OF THE WATER SOLUBLE FRACTIONS OF CROSS LAKE WATER COLLECTED BY SIZE EXCLUSION HPLC (UNCHLORINATED)

Fraction No.	Mole Wt. Range	Mole Wt. at Pk Maximum	DOC (mg/L)		THMFP* (μg/L)		THMFP/DOC †	
			$\overline{X}$	S	$\overline{X}$	S	$\overline{X}$	S
1	$31.6 \times 10^3 - 15.9 \times 10^3$	22.4×10^3	0.63	0.04	31	7	.049	.011
2	$22.3 \times 10^3 - 7.9 \times 10^3$	14.2×10^3	0.93	0.00	73	2	.078	.002
3	$19.1 \times 10^3 - 7.1 \times 10^3$	10.3×10^3	1.28	0.04	95	10	.074	.008
4	$15.9 \times 10^3 - 5.1 \times 10^3$	7.9×10^3	1.22	0.20	43	5	.035	.004
5	$6.3 \times 10^3 - 0.2 \times 10^3$	3.9×10^3	0.91	0.20	62	2	.068	.002
Average	10.5×10^3						.061	.005
Sum			4.97		304(252 as Cl)			

S = Standard deviation
* Chlorine 20 mg/L, 3 days, pH 6.5
† Units of THMFP/DOC in mg THMFP/mg C.

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fractions are shown in Figure 29. The data indicate that THMFP is evenly distributed throughout the molecular weight range of the polymer, with the possible exception of fractions 1 and 4.

Table 21 shows characteristics of water soluble fractions collected by size exclusion HPLC after chlorination of the original sample. Again, it is noted that average molecular weight has decreased as measured by the refractive index detector (compare with Table 20). Non-volatile organic halogen values in these samples were measured by the XAD method (71). The results show that the total yield of halogen in the non-volatile fraction is 260 μg/L (as Cl) as compared to a yield of trihalomethanes of 252 μg/L (as Cl). An exhaustive search for other halogen-containing volatile compounds by GC/MS revealed only small amounts (<10 μg/L) of trichloroacetic acid and other compounds (see Section 5). Thus, non-volatile compounds represent the majority of chlorinated products in the lake water.

Later experiments using the GAC/TOX procedure showed that the preponderance of non-volatile organohalides over THMs was greater than shown by the XAD procedure. Figure 30 shows the formation of trihalomethanes and TOX as measured by the LLE and GAC procedures. Final yields of THMs were 280 μg/L, whereas TOX/GAC and TOX/XAD values were 1400 and 500 μg/L (all as Cl).

Tables 22 and 23 show characteristics of size exclusion fractions of different samples of Cross Lake water. In this case only acid soluble fractions were taken, i.e. the freeze-dried residue was extracted directly with dilute nitric acid. Table 22 shows that molecular size distribution (this time determined with the UV detector) is similar to that shown in Table 20. Number average $\overline{M}_n$ and weight average $\overline{M}_w$ molecular weights of the acid soluble fraction are 3.9 x 10^3 and 8.2 x 10^3 respectively as determined by analysis of the size exclusion chromatograms. Table 22 also shows that THMFP and TOXFP are spread throughout the polymer, but on a per gram of carbon basis more formation potential is present in the lower molecular weight fractions. Taking the TOX/GAC as more accurate than TOX/XAD values, the average ratio of TOX/DOC is 0.232 mgCl/mgC (using the values for TOXFP and DOC obtained in the unfractionated material, which more closely represent conditions used in water treatment). On a molar basis this ratio is 0.080 Cl/C. If one assumes that the average molecular weight of the polymer is 3900 ($\overline{M}_n$), and that the polymer contains 50% carbon on a weight basis, the observed Cl/C ratio predicts the average polymer will contain thirteen atoms of chlorine.

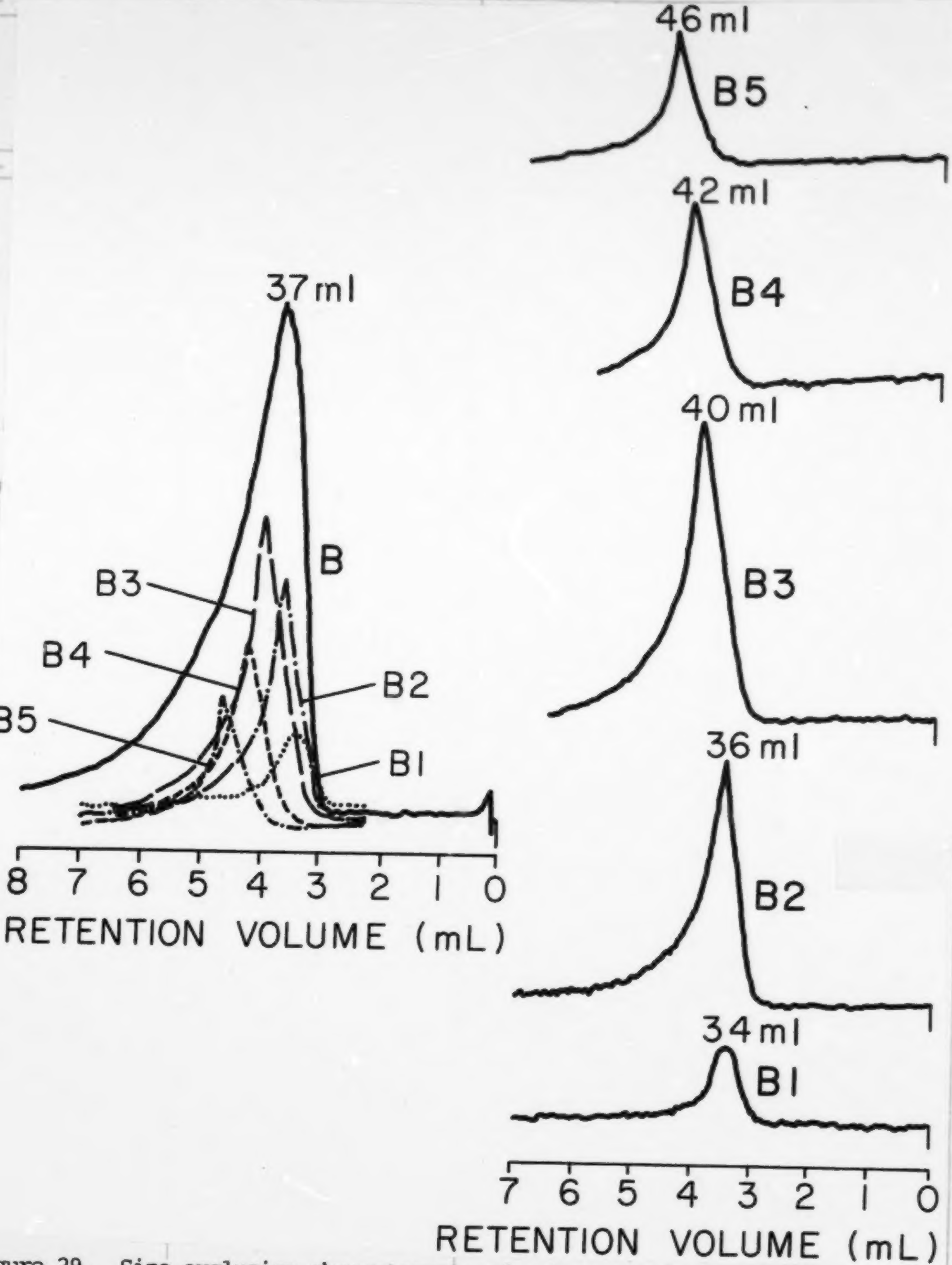

Figure 29. Size exclusion chromatograms of water soluble fractions (unchlorinated). Right: reinjected fractions; left: superposition of chromatograms of reinjected fractions over original trace.

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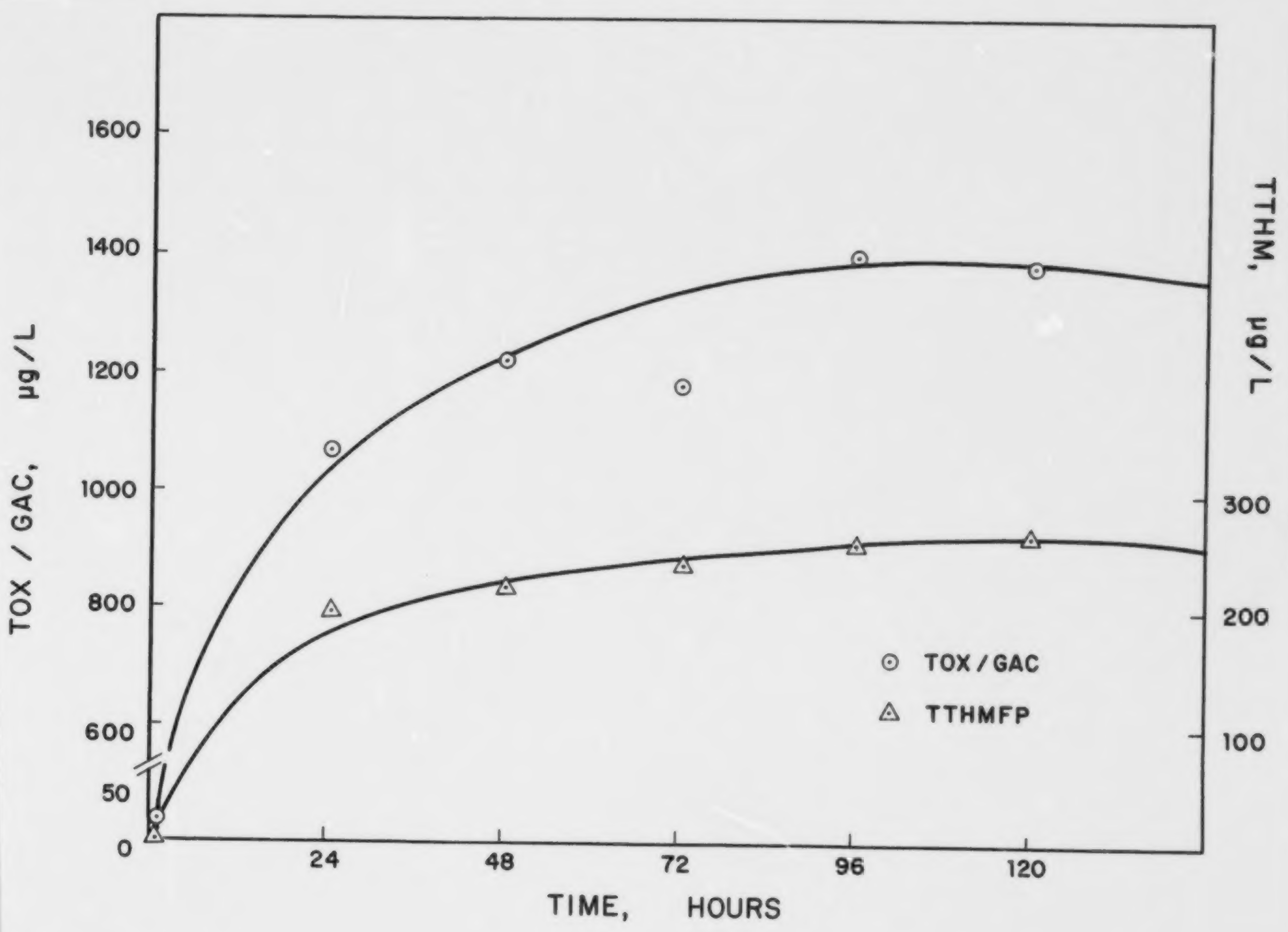

Figure 30. Trihalomethane formation potential (TTHMFP) and total organic halogen (TOX/GAC) formation potentials for Cross Lake water; 20 mg/L dose for five days.

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$$\underbrace{\frac{(3900)\quad(0.50)}{(12)}}_{\substack{\text{moles of C in}\\ \text{polymer per mole}\\ \text{of polymer}}} \quad \times \quad \underbrace{0.080}_{\substack{\text{ratio of}\\ \text{moles of}\\ \text{Cl:C}}} \quad = \quad \underbrace{13}_{\substack{\text{moles of Cl in}\\ \text{polymer per mole}\\ \text{of polymer}}}$$

Or in other words, the typical polymer has approximately 162 atoms of carbon and thirteen atoms of halogen. It should be noted that this ratio is approximately twice the value determined earlier from XAD/TOX measurements (39).

Table 24 reports the values of individual THMs formed by the chlorination of the five fractions described in Table 22. An examination of the relative molar yields of the three THMs in the five fractions suggests a somewhat lower yield of brominated THMs in the first two (high molecular weight) fractions. Schnoor and co-workers (40) suggested that brominated organics were formed from lower molecular weight precursors. However, their size fractions were collected by Sephadex gel chromatography which has been shown to be subject to severe adsorption effects when used on polymeric electrolytes (153). It should be noted that only proteins were used to calibrate their column.

Schnoor et al. (40) also report that the overall yield of trihalomethanes from their source (Iowa River water) was 2.3-7.2 µg/L of TTHM per mg/L TOC. Tables 20 and 22 show that values obtained in this work range from 35-227 µg/L of TTHM per mg/L TOC. The lower yields observed in the work of Schnoor et al. are presumably due to the shorter reaction times (10 hr. vs. 24 hr), lower chlorine dose (6 mg/L vs. 20 mg/L), and perhaps differences in sample type. It should also be noted that the pH of the Iowa samples was adjusted to 10.8 before chlorination, whereas the Cross Lake samples were run at 6.5.

Weak Anion Exchange Chromatography--

MacCarthy et al. (154) have reported recently the use of XAD resins in an HPLC mode for the separation of natural humic materials into two fractions. No structural evidence was presented but it was suggested from the values of pH at which elution occurred, that the two peaks corresponded to carboxylic acids and phenolic polymers.

Figure 31 is a chromatogram of Cross Lake water using a weak anion exchange (WAX) resin with gradient pH elution and UV detection. Weak and strong solvent refer to boric acid - borate buffer at pH 3.2 and 6.2 respectively. Also shown in Figure 31 are elution times of five low molecular weight model compounds listed in Table 25. Ionization constants K_a and elution volumes of the standards suggest that the first two

TABLE 21. CHARACTERISTICS OF THE WATER SOLUBLE FRACTIONS OF CROSS LAKE WATER COLLECTED BY SIZE EXCLUSION HPLC. (CHLORINATED*)

Fraction No.	Mole Wt Range	Mole Wt At Pk Maximum	DOC (mg/L) $\overline{X}$	DOC (mg/L) S	NVTOX** (µg/L) $\overline{X}$	NVTOX** (µg/L) S	NVTOX/DOC† $\overline{X}$	NVTOX/DOC† S
1	$31.6 \times 10^3 - 14.2 \times 10^3$	19.1×10^3	1.04	0.48	65	11	.063	.010
2	$19.1 \times 10^3 - 6.9 \times 10^3$	12.6×10^3	0.98	0.16	47	8	.048	.008
3	$15.9 \times 10^3 - 6.3 \times 10^3$	7.1×10^3	1.00	0.36	86	13	.086	.013
4	$12.6 \times 10^3 - 2.2 \times 10^3$	3.9×10^3	0.64	0.08	20	9	.031	.014
5	$7.1 \times 10^3 - 0.15 \times 10^3$	2.5×10^3	0.52	0.04	42	8	.081	.015
Average	8.2×10^3						.062	.012
Sum			4.18		260			

*Original sample chlorinated at 20 mg/L for 5 days before freeze-drying and redissolving.

**NVTOX = non-volatile TOX determined by the XAD procedure (71) after purging sample to remove THMs and other purgeable organohalides.

† NVTOX/DOX in mg Cl/mg C.

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TABLE 22. CHARACTERISTICS OF ACID SOLUBLE FRACTIONS OF UNCHLORINATED CROSS LAKE WATER SEPARATED BY SIZE EXCLUSION HPLC

Fraction	Molecular Weight Range	Molecular Wt. at Peak max.	DOC mg/L		THMFP µg/L		$\frac{\text{THMFP}}{\text{DOC}}$	--TOXFP-- XAD µg/L		--TOXFP-- GAC µg/L		$\frac{\text{TOX/GAC}}{\text{DOC}}$
			$\overline{X}$	S	$\overline{X}$	S		$\overline{X}$	S	$\overline{X}$	S	
1	$31.6\text{x}10^3$-$15.9\text{x}10^3$	$22.4 \text{ x } 10^3$	0.66	0.01	36	1	0.055	58	14	142	13	0.215
2	$22.3\text{x}10^3$- $7.9\text{x}10^3$	$14.2 \text{ x } 10^3$	1.74	0.03	118	1.1	0.068	347	15	856	110	0.492
3	$19.1\text{x}10^3$- $7.1\text{x}10^3$	$10.3 \text{ x } 10^3$	1.88	0.04	218	0.9	0.116	354	13	1064	182	0.566
4	$15.9\text{x}10^3$- $5.1\text{x}10^3$	$7.9 \text{ x } 10^3$	1.14	0.02	201	0.4	0.176	281	16	612	104	0.537
5	$6.3\text{x}10^3$- $0.2\text{x}10^3$	$3.9 \text{ x } 10^3$	0.70	C	159	1.4	0.227	187	12	340	113	0.485
Average	---	---	---		---		0.128	---		---		0.459
Sum	---	---	6.12	0.02	732*	2	---	1227*	31	3014*	262	---
Value Before Fractionation	$31.6\text{x}10^3$- $0.2\text{x}10^3$	$9.2 \text{ x } 10^3$	6.1	0.1	239*	1.6	0.039	425*	21	1419*		0.232

*TOXFP and THMFP of fractions were carried out a higher [HOCl]/(C) ratio for fractions than for the combined sample before fractionation; TOX values expressed as µg/L of chlorine.

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TABLE 23. CHARACTERISTICS OF ACID SOLUBLE FRACTIONS OF CHLORINATED CROSS LAKE WATER SEPARATED BY SIZE EXCLUSION HPLC

Fraction	Molecular Weight Range	Molecular Wt. at Peak max.	DOC mg/L		TOX-XAD µg/L*		TOX-GAC µg/L*		TOX-+ XAD / DOC	TOX-GAC+ / DOC
			$\bar{X}$	S	$\bar{X}$	S	$\bar{X}$	S		
1	$31.6 \times 10^3 - 14.2 \times 10^3$	19.1×10^3	0.82	0.1	17	4	68	13	21	0.083
2	$19.1 \times 10^3 - 6.9 \times 10^3$	12.6×10^3	0.96	0.1	81	3	157	22	84	0.164
3	$15.9 \times 10^3 - 6.3 \times 10^3$	7.1×10^3	1.74	0.1	96	10	625	136	55	0.359
4	$12.6 \times 10^3 - 2.2 \times 10^3$	3.9×10^3	0.9		72	6	410	33	80	0.456
5	$7.1 \times 10^3 - 0.15 \times 10^3$	2.5×10^3	1.0	0.2	26	5	124	15	26	0.124
Average	---	---	---		---		---		53	0.237
Sum	---	---	5.42	0.3	292	14	1384	144	---	---
Value Before Fractionation	$31.6 \times 10^3 - 0.16 \times 10^3$	8.4×10^3	5.7	0.1	346	10	771	47	61	0.135

*TOX values expressed as µg/L as chlorine.

+TOX/DOC in mg Cl/mg C.

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TABLE 24. THMs FORMED BY CHLORINATION OF ACID SOLUBLE FRACTIONS OF CROSS LAKE WATER SEPARATED BY SIZE EXCLUSION HPLC*

Fraction**	THM (µmole/L) $CHCl_3$ $\overline{X}$	$CHCl_3$ S	$CHCl_2Br$ $\overline{X}$	$CHCl_2Br$ S	CHClBr $\overline{X}$	CHClBr S	TTHMFP (µmole/L) $\overline{X}$	TTHMFP (µmole/L) S
1	0.32	0.006	0.02	0.001	N.D.	-	0.34	0.007
2	0.97	0.009	0.17	0.001	0.02	0	1.16	0.01
3	1.43	0.006	0.53	0.001	0.09	0.002	2.05	0.01
4	1.39	0.001	0.43	0.001	0.05	0.001	1.87	< 0.01
5	1.16	0.006	0.30	0.008	0.02	0.001	1.48	0.01

*No bromoform detected

**Those listed in Table 22

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peaks in Figure 32 correspond to phenols and carboxylic acid polymers as suggested by MacCarthy et al. (154). The third peak, not reported by MacCarthy et al. is of unknown molecular type.

HPLC Investigation of Municipal Wastewater

Secondary effluent from the Denton, Texas municipal wastewater treatment plant was collected before final chlorination. Characteristics of the water are shown in Table 17. Aliquots of the filtered, purged sample of 12/7/78 were chlorinated at different treatment levels and contact times. Free and combined chlorine were determined by amperometric titration according to procedures described in Standard Methods (151). Results are presented in Table 19. Based on these results, 30 mg/L chlorination level was selected for this type of water to ensure free available chlorine in the sample.

Two liters of the same filtered, purged wastewater were chlorinated at 30 mg/L level. At time intervals corresponding to 0, 1, 24, 48, 72, 96 and 120 hours, duplicate samples were collected, quenched with Na_2SO_3, and analyzed for THMs using the modified liquid-liquid extraction procedure (155). At the same time intervals, samples were collected and quenched and analyzed for TOX using the XAD procedure (71). After 120 hours the sample was purged with N_2 for one hour and samples were taken for THMs and TOX determinations. The free and residual chlorine in the sample was monitored for 48 hours. Also initial and final samples were analyzed for TOC.

Data on THMs are presented in Table 26 and data on TOX are presented in Table 27.

Examination of Table 26 shows no detectable concentration of $CHCl_3$ or TTHM in the unchlorinated purged sample. Upon chlorination and after one hour contact time 29.5 µg/L $CHCL_3$ and 48.6 µg/L TTHM were detected. This indicates that a fraction of the nonpurgeable organics react rapidly with chlorine to produce $CHCl_3$ and TTHM. Concentrations of $CHCl_3$ and TTHM showed a gradual increase and reached a plateau after 72 hours. The changes in $CHCl_3$ and TTHM concentrations after 72 hours do not seem to be statistically different.

Examination of Table 27 also indicates several important features. While the limitation of the TOX method are well recognized, the data indicate the presence of a measurable concentration (23.9 µg/L) of halogenated non-purgeable organic compounds in the purged sample before chlorination. Total THM concentration in the same sample was below the detection limits of 0.5 µg/L. Presumably these non-purgeable compounds are present in the wastewater influent.

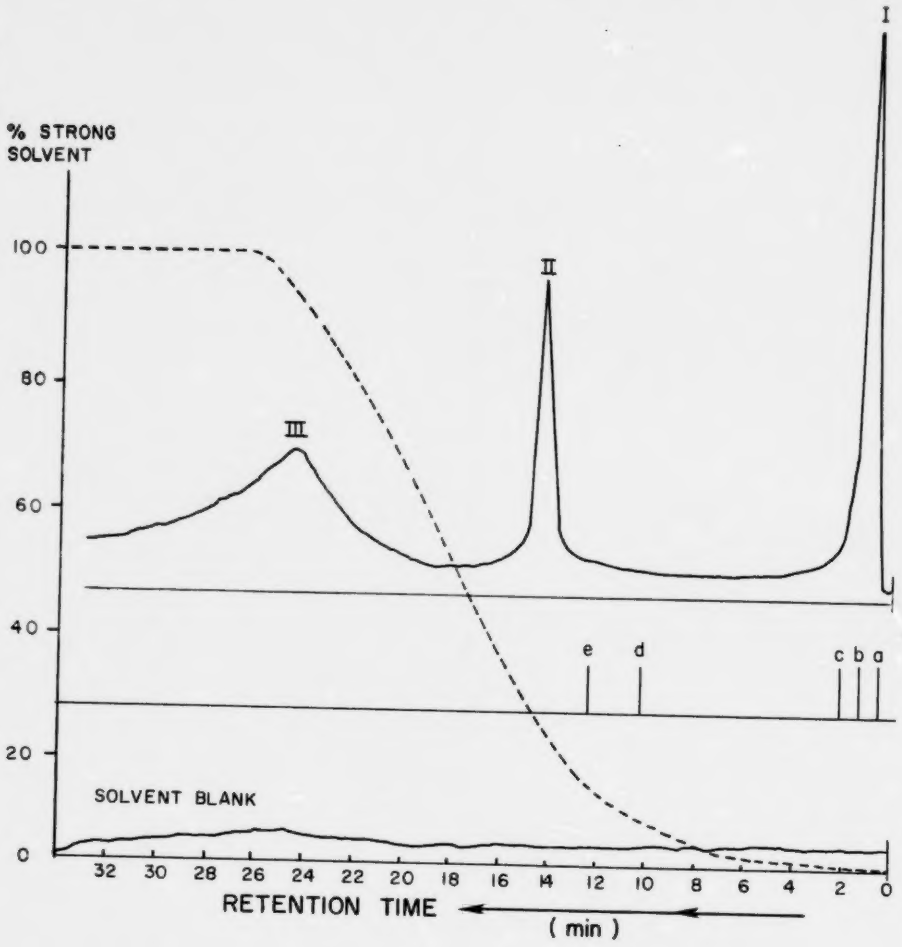

Figure 31. Weak anion exchange HPLC chromatogram of Cross Lake water, acid soluble fraction of freeze dried sample (unchlorinated). Reference compound code: a-phenol; b-3-methylcatechol; c-vanillic acid; d-2,4-dihydroxybenzoic acid; e-2,4,6-trihydroxybenzoic acid. Dotted line: solvent gradient (100%=pH 6.2; 0%=pH 3.2).

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TABLE 25. K_a AND RETENTION VOLUMES FOR STANDARD COMPOUNDS

	Compound	K_a	Retention Volume*	Retention Time**
a.	phenol	1.1×10^{-10}	3.0 mL	1.5 min.
b.	3-methylcatechol	$\sim 1.0 \times 10^{-10}$	4.0 mL	2.0 min.
c.	vanillic acid	8.3×10^{-5}	6.0 mL	3.0 min.
d.	2,4-dihydroxybenzoic acid	1.05×10^{-3}	21.4 mL	10.7 min.
e.	2,4,6-trihydroxybenzoic acid	2.1×10^{-2}	26.3 mL	13.2 min.

*N^3 concave gradient pH 3.2 - 6.2 boric acid - borate buffer in 25 minutes.

**See figure 31.

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TABLE 26. TRIHALOMETHANE FORMATION POTENTIAL DATA

Sample Designation	μg/L $CHCl_3$ $\overline{X}$	 $CHCl_3$ S	 $CHCl_2Br$ $\overline{X}$	 $CHCl_2Br$ S	 $CHBr_2Cl$ $\overline{X}$	 $CHBr_2Cl$ S	 $CHBr_3$ $\overline{X}$	 $CHBr_3$ S	 TTHM $\overline{X}$	 TTHM S
Unchlorinated Unpurged	4.1	0.31	1.1	0.06	0.85	0.11	<0.5	-	6	0.48
Unchlorinated Purged	<0.5	-	<0.5	-	<0.5	-	<0.5	-	<0.5	-
Chlorinated 1 h	29.5	2.96	10.8	1.25	5.3	0.57	<0.5	-	48.6	0.35
Chlorinated 24 h	60.4	4.50	27	0.51	10.7	0.21	<0.5	-	98	4.80
Chlorinated 48 h	67	4.90	29.2	2.60	11.3	1.10	0.76	0.18	108.3	8.82
Chlorinated 72 h	77.9	3.78	29.9	1.46	11.3	0.65	0.89	0.06	119.9	5.96
Chlorinated 96 h	74.3	1.45	29.1	0.50	11.3	0.22	1.0	0.09	115.6	2.26
Chlorinated 120 h	72.1	3.08	28.9	1.19	11.5	0.43	1.0	0.01	113.5	4.70

$\overline{X}$ Mean of duplicate analysis

S Standard deviation

TTHM Total trihalomethane

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Upon chlorination the TOX values also increased after one hour contact time indicating the instantaneous formation of halogenated organic compounds from the non-purgeable precursors present in the sample. The TOX formation potential follows essentially the same pattern as the TTHM. Tables 26 and 27 show that throughout the chlorination process, non-purgeable organic halogen which is XAD-adsorbable is equal or greater than the halogen in the form of THMs. As noted in the first part of this section, XAD adsorption does not measure total organic halogen (149); thus, the TOX value of the non-purgeable fraction may be assumed to outweigh the purgeable fraction (THMs) by at least a factor of 2 to 3.

TABLE 27. NON-PURGEABLE TOX FORMATION POTENTIAL OF A SECONDARY MUNICIPAL WASTEWATER EFFLUENT

Sample Designation	(µg/L) $\bar{X}$	S
Procedure Blank	8.8	1.2
Unchlorinated Unpurged	28.5	3.8
Unchlorinated Purged	23.9	5.2
Chlorinated, 1h	52.7	2.2
Chlorinated, 24h	187	5.3
Chlorinated, 48h	211.8	13
Chlorinated, 72h	233.7	11.2
Chlorinated, 96h	221	12.3
Chlorinated, 120h	205.3	13.4

$\bar{X}$ Mean of two injections 20 µl each

S Range x 0.89

Examination of Freeze Dried Concentrates--

Four hundred mL of filtered, purged wastewater sample was freeze dried under vacuum. A fluffy residue was left upon the evaporation of the water. The freeze dried (FD) residue was separated into three portions and dissolved in organic-purified, deionized water (WATER SOLUBLE FRACTION), 0.1 N HNO_3 (ACID SOLUBLE) and 0.1 N NaOH (BASE SOLUBLE). The measured pH of the ACID and BASE fractions were 2 and 11 respectively. The dilute acid was found to dissolve almost all of the residue.

The supernatent solutions from each of the solubility tests were filtered through 0.45 μ pore size filter and the base fraction was neutralized to pH 7 with few drops of 6 N HNO_3*. One mL of the H_2O soluble fraction was qualitatively chlorinated with about 50 mg/L Cl_2 at contact time of one hour. All filtered fractions of the FD solubility tests were subjected to size exclusion HPLC under the conditions described in the experimental part of this section. Figure 32 shows the size exclusion chromatograms of the unchlorinated and chlorinated water soluble fractions. The chromatogram of the chlorinated sample shows a shift of peak #1 towards the lower molecular weight end and a formation of peak #II beyond the total permeation volume. Since the sample was not purged, it is likely that peak #II corresponds to the volatile halogenated compounds which partition with the glycophase coating on the silica beads. Earlier experiments showed that peak #II could be removed by purging the sample before HPLC.

The HPLC effluent corresponding to peak #I in both the chlorinated and unchlorinated fractions (Figure 32) was collected and subjected to TOX analysis by direct injection of 20 μL into the Dohrmann microcoulometer. Presumably these fractions contain no inorganic halide ions since these are eluted along with the small molecules at the total permeation volume. The TOX values for the HPLC effluent of the unchlorinated and chlorinated samples were 23.3 μg/L and 147 μg/L, respectively. These results are based on the original volume of the sample before freeze drying.

Figure 33 shows the size exclusion chromatograms of the acid and base soluble fractions. The size exclusion chromatograms of the H_2O, acid and base soluble fractions were analyzed for molecular size distribution based on a standard curve of sodium polystyrene sulfonate and proteins in molecular weight ranging from 1.6×10^3 to 45×10^3 as described before. The results are presented in Table 28. The water soluble fraction showed an apparent molecular weight ranging from 0.63×10^3 to 15.85×10^3 with an average of 2.99×10^3. The molecular weight

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*pH adjustment to 7 is necessary in order to perform size exclusion HPLC on the glycophase/silica column.

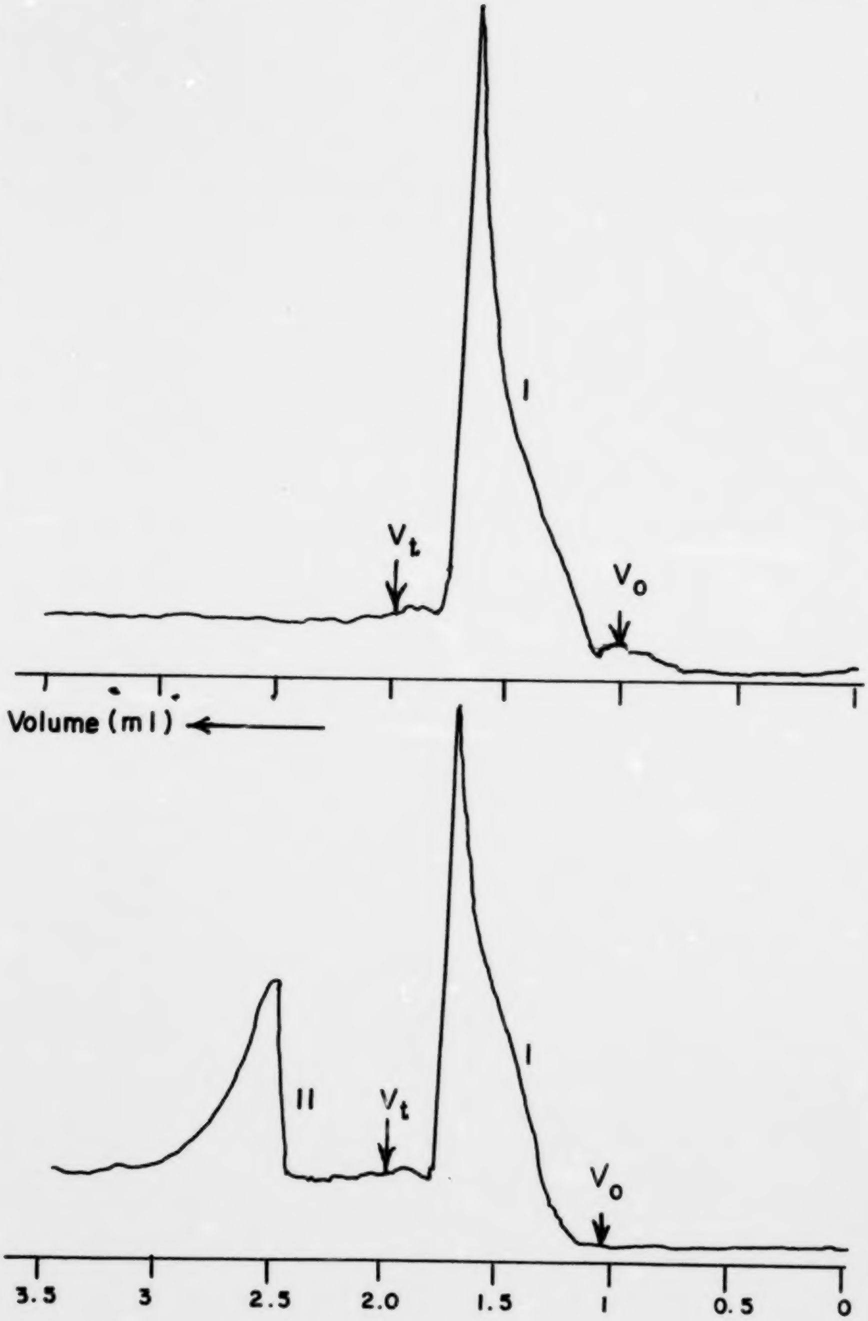

Figure 32. Size exclusion chromatograms of water soluble fraction of freeze dried Denton municipal wastewater. Top: unchlorinated; bottom: chlorinated.

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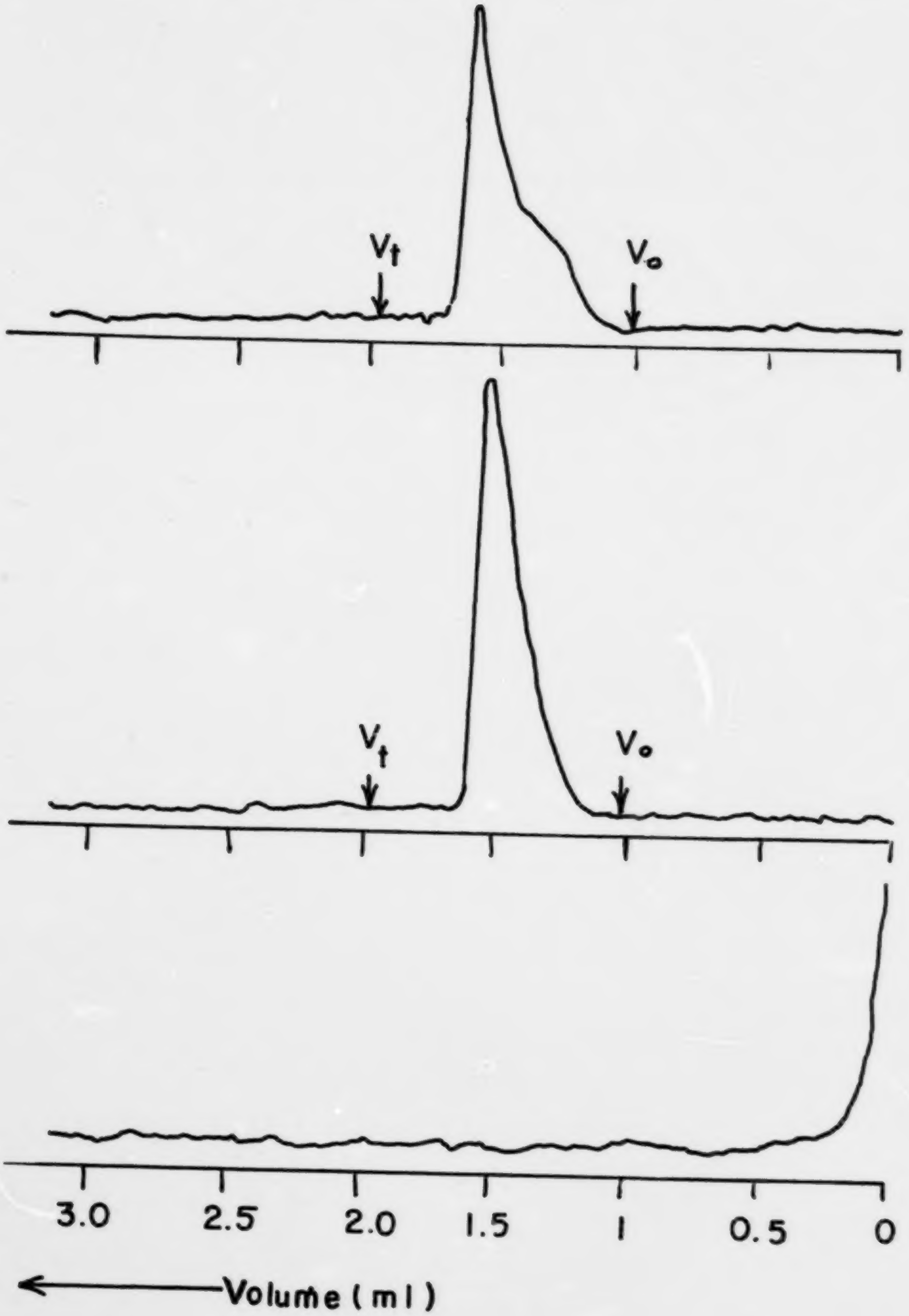

Figure 33. Size exclusion chromatograms of acid (TOP) and base (BOTTOM) soluble fractions of freeze dried Denton municipal wastewater.

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TABLE 28. MOLECULAR SIZE DISTRIBUTION FOR THE FREEZE DRIED UNCHLORINATED SAMPLE: DENTON SECONDARY WASTEWATER

Water Soluble Fraction		Acid Soluble Fraction (pH2)		Base Soluble Fraction (pH11)	
Mean Mole Wt.	%	Mean Mole Wt.	%	Mean Mole Wt.	%
15850	3.2	25120	1.43	25120	1.2
8910	6.96	15850	5.71	15850	3.1
5010	10.66	8913	13.57	8913	13.4
3160	15.16	5012	17.14	7079	30.48
1990	19.67	3162	26.42	3981	39.6
1000	43.0	2239	33.57	3162	12.19
630	1.2	1995	2.14		
Mean 2990		4960		6100	

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of 1000 represents the highest percentage of the water soluble fraction. The acid soluble fraction showed an apparent molecular weight ranging from 2.00 X 10^3 to 25.12 X 10^3 with an average of 4.96 X 10^3. Apparent molecular weights of 2.24 X 10^3 and 3.16 X 10^3 represent a substantial fraction, ca. 60 percent, of the acid soluble fraction. The base soluble fraction showed an apparent molecular weight ranging from 3.16 X 10^3 to 25.1 X 10^3 with an average of 6.10 X 10^3. Apparent molecular weights of 3.98 X 10^3 and 7.08 X 10^3 represent 70 percent of the base soluble fraction.

Several points need to be considered regarding the interpretation of the freeze drying experiment. One relates to the results of the solubility tests. The formation of an only slightly water soluble freeze dry residue is not surprising. Organic compounds in the sample could be in the colloidal form as well as in true solution. At the low temperature of the freeze drying process and in the presence of a complex matrix of inorganic salts, agglomeration of colloidal particles is likely to occur. Chelation between organic ligands and metal ions to form water insoluble molecules is another possibility.

Considering these factors, distilled water is expected to solubilize only a small fraction of the freeze dry residue. Dilute HNO_3 is expected to interact and solubilize the inorganic components of the residue which consist essentially of the CO_3^{2-}, HCO_3^-, Cl^-, SO_4^{2-} and PO_4^{3-2}, of the Na^+, Ka^+, Ca^{2+} and Mg^{2+}. Dilute HNO_3 will also dissolve basic organic compounds such as nitrogenous compounds, substitute the metal ion in the organic complexes with H^+, and affect partial hydrolysis of the organic esters. Dilute NaOH is expected to precipitate the hydroxides of the divalent and trivalent cations in the residue. Also, it will dissolve organic acids and affect partial hydrolysis of the esters.

Molecular size distribution data presented in Table 28 seems to confirm the previous discussion to some extent. The water soluble fraction consisted essentially of smaller molecules. The acid and base soluble fractions contained higher percentages of the larger molecules.

There are some limitations to the molecular weight distribution data. For one, the molecular weights are based on the retention volume of compounds of known structure and exact molecular weights. The compounds under investigation are yet to be identified and their accurate molecular weights are yet to be determined.

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CONCLUSION

The study of the non-volatile components of a municipal drinking water source and a municipal wastewater reported in this section is by no means a completed story. The results are only fragmentary and should be viewed mainly as a portent of studies to come in the future. Undoubtedly more elegant separations and spectroscopic methods will be developed to assist in the elucidation of these materials whose complex, polymeric structures make them so intractable.

Nonetheless, the studies reported here show that a substantial amount of carbon-bound halogen occurs in the non-volatile matrix which remains largely unchanged in the drinking water or wastewater processes which are in common use. The need for further study of the environmental fate and effects of these compounds is implied, as well as further investigations of their molecular structures before and after disinfection processes.

SECTION 8

ANALYSIS OF VOLATILE CHLORINATED ORGANICS IN WATER BY LIQUID-LIQUID EXTRACTION

INTRODUCTION

One of the most important classes of chlorinated organic compounds generated during water chlorination appears to be the volatile chlorinated organics (VCOs). The four most prominent compounds in this class are the trihalomethanes (THMs): chloroform, bromodichloromethane, chlorodibromomethane and bromoform. They are important because recent data indicate they comprise a significant portion of the total chlorinated organic material generated by water chlorination (156), and because the THMs are all known or suspected to have toxic and/or carcinogenic (157) potential.

The relationship between water chlorination and the formation of these VCOs has only recently been recognized. Glaze *et al*. (36) were the first to demonstrate that VCOs could be formed by the action of chlorine in water. They reported chloroform as a product of the chlorination of municipal wastewaters. However, the analytical techniques which they used were not designed to accurately quantitate such highly volatile pollutants. J. J. Rook (37) adapted a "closed system" headspace analytical procedure originally used in the flavor industry, which was a more appropriate technique for volatile analytes. He later used this technique to clearly demonstrate the formation of all of the THMs during drinking water chlorination (158).

Concern about the presence of VCOs increased as more studies confirmed their occurrence in water supplies and drinking water in other geographical areas. In 1974, a new analytical technique was developed by Bellar, Lichtenberg, and Kroner (88), which offers several advantages over the headspace technique. In the Bellar method, 5 ml sample aliquots are purged with inert gas which entrains the VCOs and transports them to a trap containing an adsorbent resin, usually Tenax GC. Following adsorption, the gas flow is reversed, the trap heated, and VCOs desorbed onto the head of a GC column for subsequent analysis. The headspace procedures used by Rook requires larger samples for analysis necessitating bulky sampling equipment and laboratory glassware. Also, the Bellar method requires only 15 minutes for the concentration portion of the analytical procedure versus 12 hours for the analytes to equilibrate between the liquid and gas phases in Rook's headspace technique.

As a result of these advantages, the Bellar method was adopted by the United States Environmental Protection Agency, and subsequently by VCO analysts in general. This method was used by the USEPA for the National Organics Reconnaissance Survey (159) and the National Organics Monitoring Survey (156) which showed the presence of various combinations and quantities of VCOs in all of the eighty U.S. cities sampled during the survey.

The impact of these studies on the water treatment field is already significant and will probably become more important in the near future. The role of chlorine in the water and wastewater treatment field is undergoing extensive reevaluation because of the potential harmful side effects of chlorinated organics such as the THMs which are produced in the process. Mandatory VCO monitoring programs and maximum allowable limits for THMs in drinking waters have recently been promulgated by the USEPA (160).

These developments imply that great numbers of VCO analyses will soon have to be run by water treatment facilities as well as regulatory and surveillance authorities. Much manpower and equipment will be required if the Bellar method is used for routine screening. The alternative is to develop new and even more streamlined analytical techniques. Nicholson (76) recognized this and underscored the importance of an analytical method which can ". . . handle large numbers of samples in a relatively short period of time" as well as be "highly specific for halogen-containing compounds".

In accordance with these requirements, two new analytical techniques have been developed. The first, developed by Nicholson (76), is a direct aqueous injection technique (DAI). This technique takes advantage of the high sensitivity of the electron capture gas chromatographic detector to circumvent any concentration step. The second technique is the liquid-liquid extraction (LLE) method (161). It utilizes a closed system extraction step followed by electron capture gas chromatography. This chapter describes in detail the LLE method and compares it to the other two contemporary methods, the Bellar purging method and the DAI procedure.

Important parametric critiques of the headspace, the Bellar, and the LLE analytical methods have been published which have led to modification in equipment and procedures. Kaiser and Oliver (162) have reported a miniaturized headspace system which only requires sixty milliliters of sample. Headspace analyses can be performed in approximately forty-five minutes using this system. The equilibration step is carried out at a reduced pressure which allows equilibration to occur in only thirty minutes. The small sample size requires the use of the more sensitive electron capture detector, but this makes Kaiser and Oliver's headspace method more specific for halocarbons than Rook's survey procedure. Kaiser and Oliver's paper also reports the results of their study of the important parameters which control the headspace procedure. Although these data were collected using their new miniaturized headspace system, most of them are universally applicable to headspace procedures.

Kus, et al. (163) have published a definitive analysis of the important parameters which control the performance of the purging methods. Although their study included the evaluation of several purging systems of various configurations, they concluded that the Bellar system was the most effective. Therefore, the majority of the data reported was collected using the Bellar apparatus. These data will be the primary ones cited as representative of the purging method in comparing it to the liquid-liquid extraction procedure described in this section.

Liquid-liquid extraction techniques have been and still are the classical means of concentration of trace organic compounds in water. The most important area in which these techniques are currently being used is in the analysis of pesticides, herbicides, and fungicides (164). These procedure typically call for a series of organic extractions followed by a several-fold evaporative concentration of the extracting solvent before analysis. Such extensive handling and transferring of the water sample and extracting solvents might be acceptable for classical applications, but new interest in much more volatile analytes requires considerable modification of the classical procedures to avoid losses due to volatilization. Grob and Grob (165) were among the first investigators to recognize this. In their liquid-liquid extraction procedure, a high ratio of water to organic solvent is used which eliminates the need for concentration, and the organic layer is sampled directly out of the extraction vessel for chromatographic analysis which reduces the losses due to sample handling and transferring.

Since the LLE method developed in this work was first reported (161), two other liquid-liquid extraction procedures have been published by Richard and Junk (166) and Mieure (167), respectively. Although neither procedure utilizes a closed extraction system, both procedures place heavy emphasis on the importance of the careful handling of both the water sample and the organic solvent in order to avoid losses due to volatilization.

EXPERIMENTAL

Samples for the LLE, the Bellar and the DAI procedures were collected and handled in identical manners. It has been recognized (150) that samples must be collected so as to avoid contact with air bubbles within the sampling container which could cause analyte losses due to water/headspace partitioning. Therefore, samples were collected in 125 ml serum bottles filled to overflowing and then sealed with teflon-lined silicone septa crimped in place by an aluminum outer sleeve (Figure 34). Before sampling, the bottles were cleaned with chromic acid, water, acetone, and then dried in an oven at 165°C for several hours. After sampling was completed, the bottles were transported to the laboratory for analysis. If more than a few hours had to elapse between sample collection and analysis, the sample bottles were chilled with ice and then warmed to room temperature before analysis.

Two reagents were added to the samples at the time of collection. Sodium sulfite (Baker analytical grade) was added as a chlorine reducing agent in varying amounts depending on the anticipated chlorine residuals. This reduction of residual free chlorine to inorganic chloride prevented chlorination reactions from occurring subsequent to sampling (150). A buffer was also added to avoid possible extraction anomalies related to pH effects (1.2 ml of a buffer prepared from a 2:3 mixture of 1.0 M NaH_2PO_4 and 1.0 M Na_2HPO_4 pH 6.5). Although no pH effects were observed during preliminary tests on spiked samples, it was felt that not all possible matrix variations could be anticipated; thus, the decision was made to continue to use the buffer despite the lack of any data indicating its usefulness.

LLE Procedure

The LLE method basically involves a specialized closed system organic solvent extraction procedure followed by chromatographic separation and analysis. A schematic representation is shown in Figure 35.

Pentane Extraction Procedure--

Normal pentane (Fisher, pesticide grade) is used as the extracting solvent. Chromatographic analysis of the pentane prior to use in the LLE procedure usually shows this grade of solvent to be of adequate quality as received. If purification is necessary, it is effected by fractional distillation from sodium metal or by passing through chromatography reagent grade alumina. The internal standard, 1,2-dibromoethane (Aldrich, reagent grade), is distilled and added to the solvent at a concentration of approximately 20 μg/l.

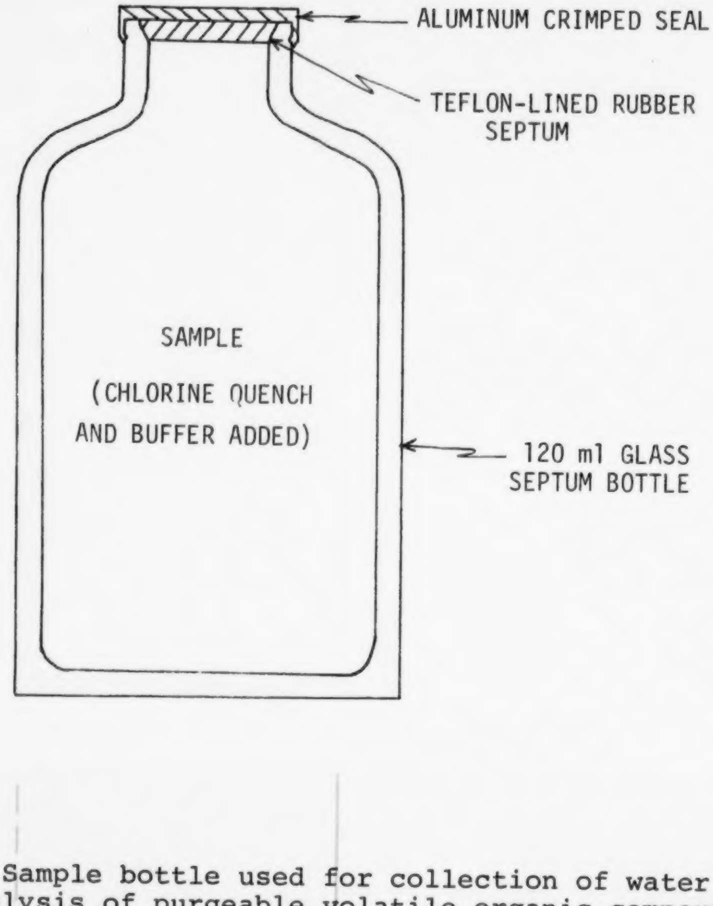
ALUMINUM CRIMPED SEAL
TEFLON-LINED RUBBER SEPTUM
SAMPLE
(CHLORINE QUENCH AND BUFFER ADDED)
120 ml GLASS SEPTUM BOTTLE

Figure 34. Sample bottle used for collection of water samples for analysis of purgeable volatile organic compounds.

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Three ml of this solvent/internal standard mixture is added in the manner shown in Figure 36 to the 125 ml water sample using two 10 cc syringes. One syringe contains the solvent mixture while the other is empty. As the solvent mixture is injected into the inverted sample bottle, it rises to the top of the bottle and an equivalent amount of water is displaced into the empty syringe. The sample bottle is then strapped to the surface of a platform gyratory shaker (Junior Orbit Shaker, Labline Instruments, Inc.) and shaken at a speed of 400 rpm/for twenty-five minutes. After shaking, the samples are ready for immediate analysis.

Chromatographic Analysis--

A two to five microliter pentane aliquot is removed through the silicone septum with a Hamilton 801 ten microliter syringe. This aliquot is then injected into a Tracor 560 gas chromatograph equipped with a ^{63}Ni linearized electron capture detector. The glass chromatographic column is 183 cm by two mm I.D. and is packed with ten per cent squalane on 100/120 mesh Supelcoport (Supelco Inc.). The carrier gas is a 95/5 per cent argon/methane mixture. The column flow rate is 20 ml/min with 60 ml/min of makeup gas (the same argon/methane mixture) added to the GC column effluent to improve detector performance. The respective oven temperatures are: injector 100°C, column 66°C, detector 300°C. An electronic digital integrator (Supergrator, Columbia Scientific Instruments) is used for quantitation. Chromatograms are recorded on a Perkin Elmer 56 strip chart recorder.

Direct Aqueous Injection

The same chromatograph, integrator, and recorder are used for this procedure as were used for the LLE procedure described above. The glass GC column used is 122 cm by two mm I.D. and is packed with Chromosorb 102 60/80 mesh, a polystyrene/divinylbenzene copolymer adsorbent. The injector, column and detector temperatures are 175°C, 135°C, and 300°C, respectively. The procedure followed is to remove a three to five microliter water aliquot directly from the VOA sample bottle and inject it into the chromatograph.

Bellar Purge and Trap Method

A schematic diagram of the procedure used in this laboratory is shown in Figure 37. The procedure is essentially the same as that outlined by Bellar, et al. (88) with three important modifications (refer to Figure 37):

1. A liquid chromatographic sample loop injector (Altec, Inst. # 5U8031) is used to accurately introduce reproducible aliquots of water samples into the purging device.

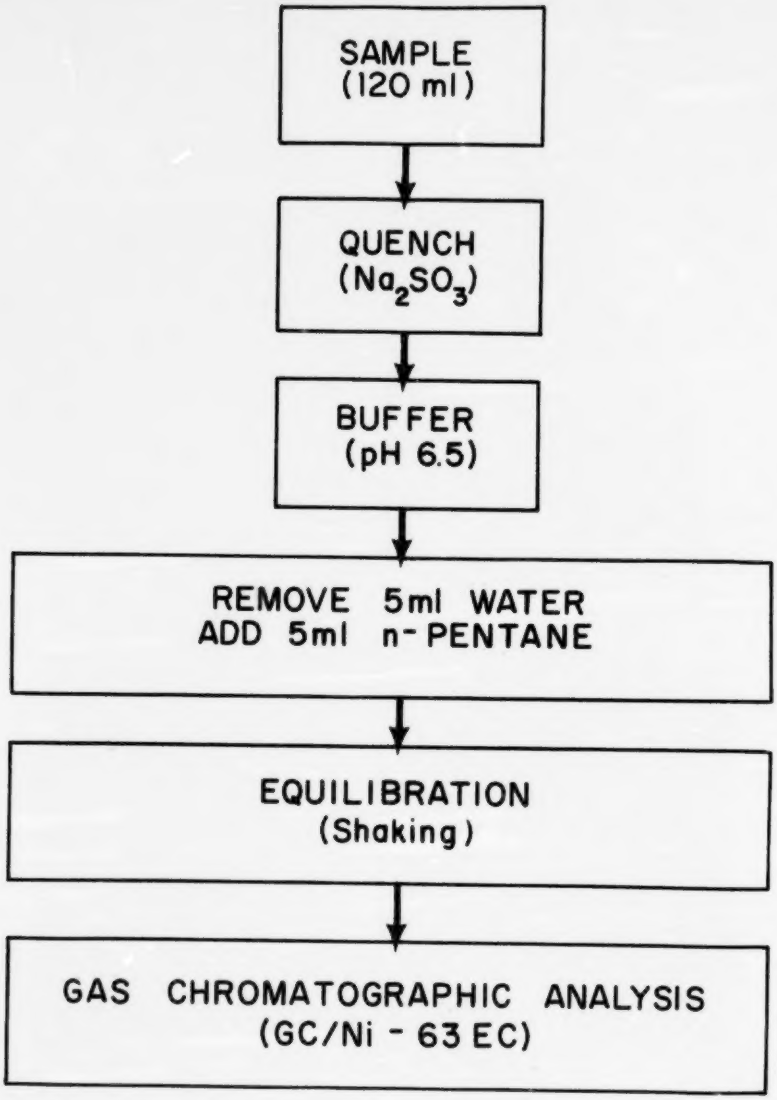
SAMPLE
(120 ml)
QUENCH
(Na_2SO_3)
BUFFER
(pH 6.5)
REMOVE 5ml WATER
ADD 5ml n-PENTANE
EQUILIBRATION
(Shaking)
GAS CHROMATOGRAPHIC ANALYSIS
(GC/Ni - 63 EC)

Figure 35. Scheme for analysis of volatile organics by liquid-liquid extraction.

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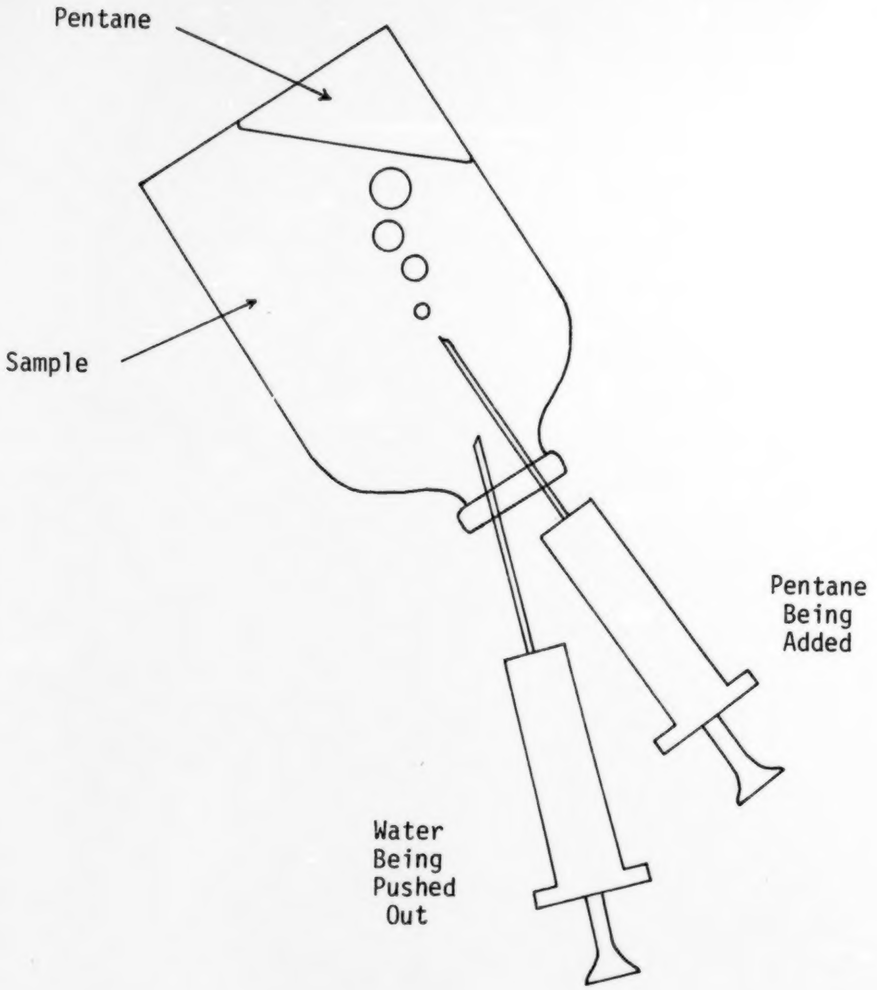
Pentane
Sample
Pentane Being Added
Water Being Pushed Out

Figure 36. Procedure for removal of water and addition of pentane for volatile purgeable organic analysis.

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2. The analytical column serves as the adsorbing trap. Thus the VCOs are purged directly onto the chromatographic column eliminating a significant source of erratic results.

3. The Chromosorb 102 analytical column which is used allowed the separation of several less significant analytes such as carbon tetrachloride and 1,2-dichloroethane which could not be separated using Bellar's original analytical column.

Purging Procedure--

The 5.5 ml sample injector loop is filled to overflowing with the water sample using a 20 cc syringe. The valve is switched to the inject position and the sample forced to flow into the purging device by the pressure of the helium carrier gas. As the pressure continues to build, the helium purges the volatile analytes from the water and sweeps them into the analytical column which is cooled to room temperature. A carrier gas bypass valve allows the purging equipment to be circumvented during chromatographic analysis thus obviating possible problems due to water-saturated carrier gas.

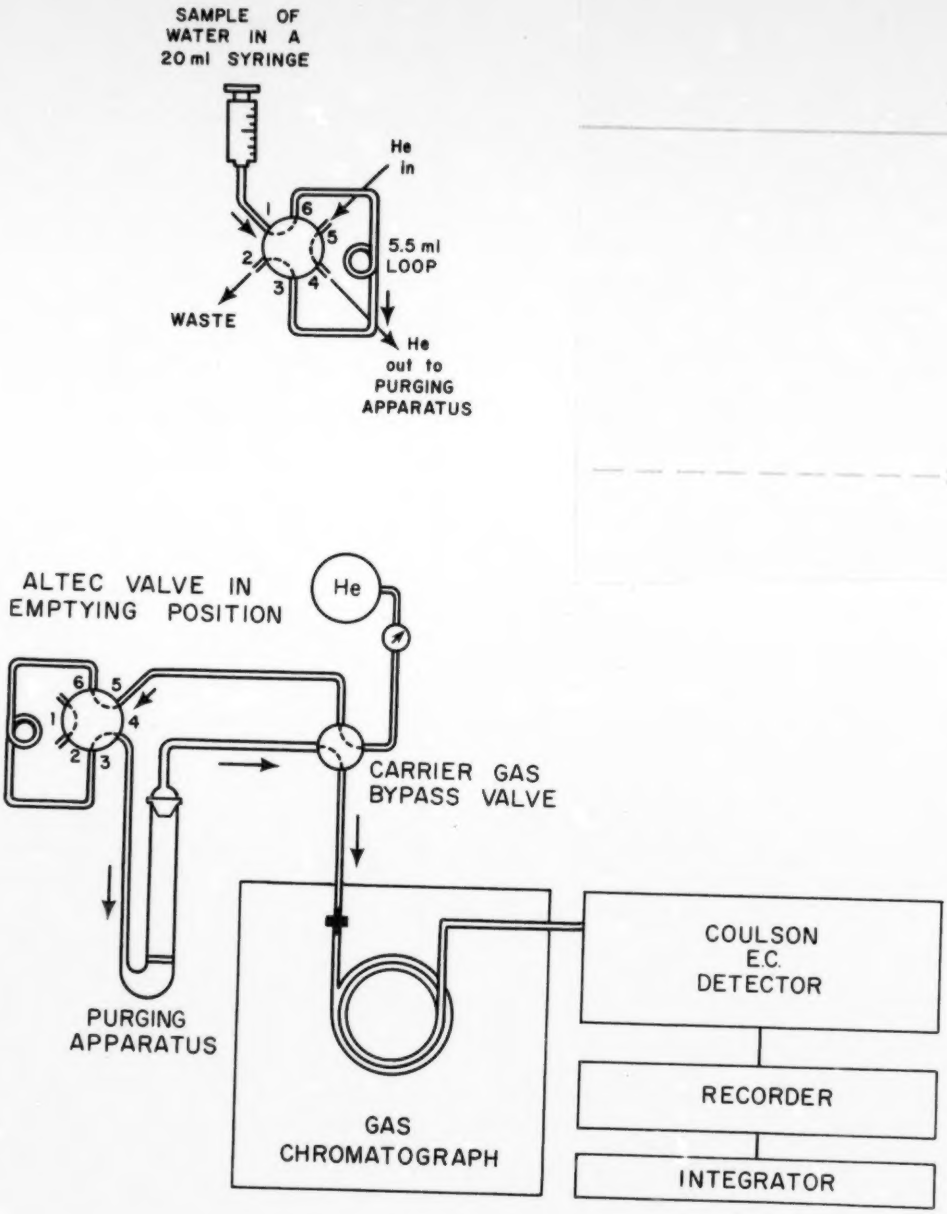
Chromatographic Analysis--

The gas chromatograph is a Hewlett Packard 3700 equipped with a Coulson Electrolytic Conductivity Detector (CECD-Tracor Inst., Inc., Austin, Texas). The glass chromatographic column is 122 cm by 2 mm I.D. and contains Chromosorb 102 (60/80 mesh). The helium carrier gas flow is 20 ml/min. The injector temperature is 200°C, and transfer lines and venting valve leading to the CECD are at 275°C. After sample introduction, the GC column oven is programmed ballistically from ambient temperature to 60°C, then from 60° to 220° at 8°/min. The CECD is operated in the reductive mode with 60 ml/min of hydrogen added to the GC effluent prior to the influent end of the pyrolysis furnace which is at 850°C. The recorder and integrator are the same as those used in the LLE and DAI procedures.

RESULTS AND DISCUSSION

A comparison has been made in the laboratory between the LLE method and the Bellar method for VCO analysis. Although the Direct Aqueous Injection technique was also evaluated relative to the LLE method, the comparison was less extensive than with the Bellar method for reasons described below.

Typical chromatograms are shown in Figures 38-40 for the three analytical methods. The concentration of chloroform in all samples was about 40 ppb. Other analyte concentrations were adjusted using the appropriate response factors (shown in Table 29) to produce peak heights approximately equal to that of chloroform. It is important to note that the response


SAMPLE OF WATER IN A 20 ml SYRINGE
He in
1
6
5
5.5 ml LOOP
2
3
4
WASTE
He out to PURGING APPARATUS
ALTEC VALVE IN EMPTYING POSITION
He
6
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CARRIER GAS BYPASS VALVE
PURGING APPARATUS
GAS CHROMATOGRAPH
COULSON E.C. DETECTOR
RECORDER
INTEGRATOR

Figure 37. Modified purge and trap apparatus with liquid sample loop injector.

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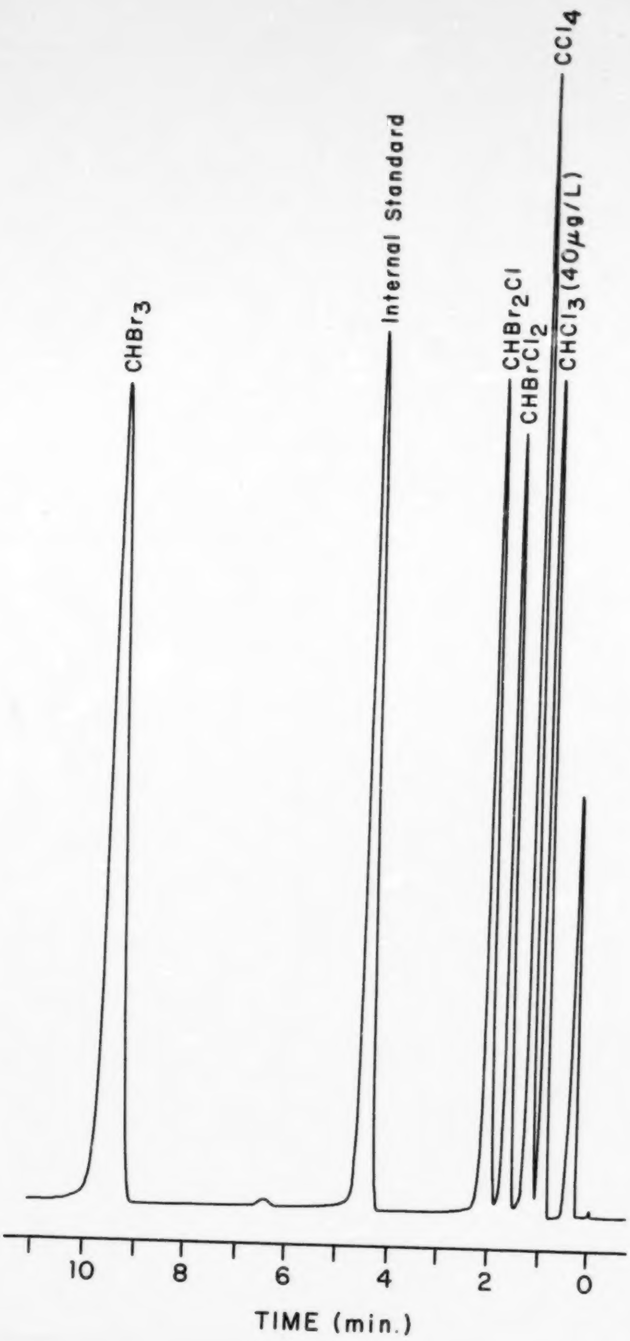

Figure 38. Electron capture gas chromatogram of VCOs from pentane LLE extraction (conditions in text).

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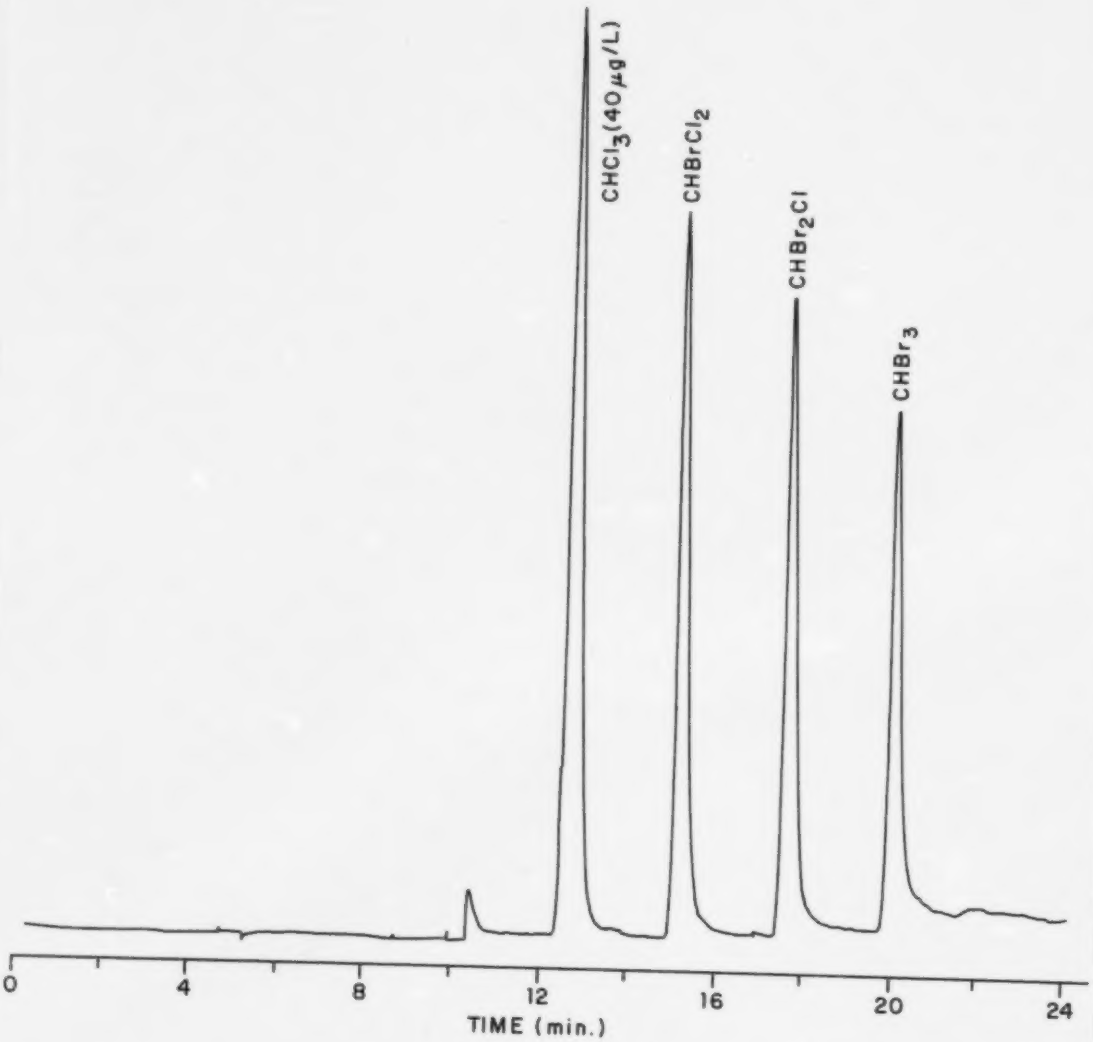

Figure 39. Coulson electrolytic conductivity gas chromatogram of VCOs from modified purge/trap procedure (conditions in text).

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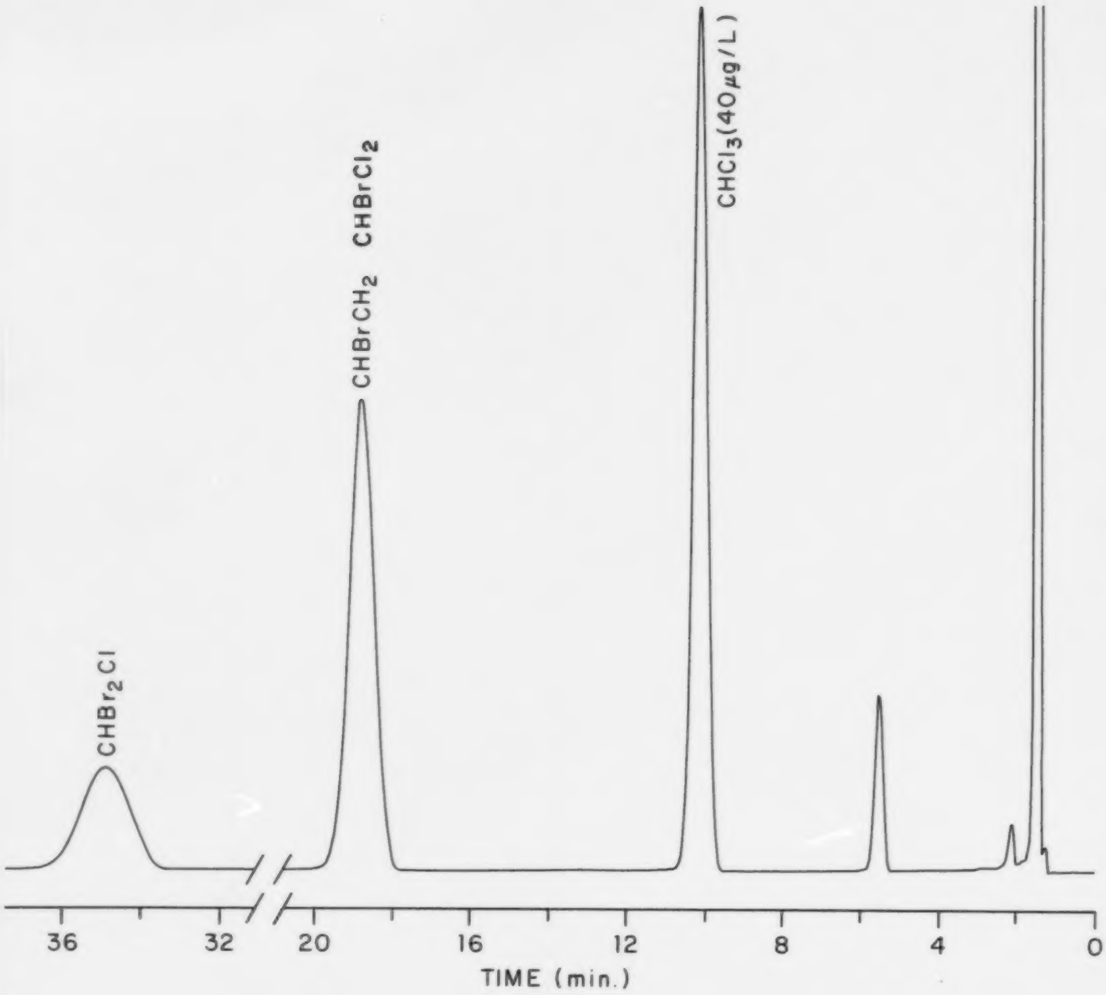

Figure 40. Electron capture gas chromatogram of VCOs by direct aqueous injection (conditions in text).

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factors reflect concentrations which produce similar peak heights as opposed to equal areas. This approach was used for two reasons: firstly, the classical (though less reliable) quantitation of trace analytes by gas chromatography has usually been performed by manually measuring peak heights to calculate concentrations. This is the procedure used by Bellar (88) in his first report, and is the procedure still being widely used today. Secondly, signal to noise ratios (S/N) are related to peak heights rather than areas. Since the minimum detectable limit is a function of the S/N, it is therefore also a function of peak height.

The digital electronic integrator used in our laboratories measures both peak heights and areas automatically. Therefore, either variable can be selected as the basis of quantitation. The estimates of quantitative precision are shown in Table 30. For precision studies, the analyte concentrations were all adjusted to an approximate 20 to 200 S/N ratio. Therefore, these precision values probably approach the optimum which can be produced for each respective method. Of particular interest is the fact that the internal standard quantitation method used for the LLE procedure produced values roughly comparable to the values for the Bellar method which used the usually less precise external quantitation method. This is probably due to the use of the liquid chromatographic sample loop injector in the Bellar method which allows good reproducibility of sample size for successive analyses. The precision for all techniques is generally at or below the 5 per cent level which is acceptable precision for a trace analytical technique of this type.

The minimum detectable limits (MDL) were determined by analyzing increasingly dilute standard mixtures until a signal to noise ratio of approximately 2 was reached. The resulting values are shown in Table 31. The data indicate the LLE technique to be generally more sensitive than the Bellar or DAI methods. However, it should be noted that the MCL values for the Bellar method were determined using the Coulson detector. Recently, this detector has been replaced with the Hall detector which is more sensitive by at least one order of magnitude. Hence the Bellar method is at least as sensitive as the LLE method and in the case of compounds such as 1,2-dichloroethane the Bellar method is much more sensitive. The MCL values of the DAI method generally are greater by an order of magnitude than either of the other two methods.

LLE Parameters

Solvent Selection--

Pentane is used as the organic extraction solvent for several reasons. The Fisher pesticide grade commercial

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pentane is usually pure enough to use as received. It is volatile enough to separate easily from the chloroform peak, the first peak of analytical interest. It has a low electron capture response producing little or no solvent front. It is highly insoluble in water; and it has a very favorable distribution coefficient versus water for the analytes of interest (Table 32).

As noted earlier, Grob and Grob (165) first developed a survey liquid-liquid extraction process using pentane as a solvent. Their system uses 200 μl of pentane to extract 900 ml of water. While this procedure seems to work reasonably well in their hands, it certainly requires a highly skilled technician with much experience in the technique to achieve reproducible results. The procedure also takes more person-hours as it requires manual shaking of the extraction vessel for maximum efficiency. Grob and Grob indicate a loss of extraction efficiency for more volatile components which might be attributed to volatilization of such analytes due to the headspace present in their extraction apparatus.

Richard and Junk (166) also selected pentane as a solvent for essentially the same reasons cited above. For part of their work, a flame ionization detector was used which required more polar chromotographic columns for greater separation of the solvent front from the chloroform peak. This requirement was also observed in this work when using a GC/MS system for analysis.

Mieure (167) used methyl cyclohexane as a solvent. Although this solvent has a boiling point of 101°C, he indicates that it separates adequately from the chloroform peak. We observed interferences with such solvents as hexane (b.p. 69°C) for our chromatographic system, and therefore, did not try higher boiling solvents.

Extraction Apparatus--

The extraction apparatus used in the LLE procedure is the only closed extraction system having no headspace in contact with the pentane/water matrix. The three other extraction systems cited above do have a headspace in their respective extraction steps. While this may not be a rigid requirement for VCO analysis, one advantage of the closed LLE procedure is that emulsion problems seem to be eliminated due to the relatively docile nature of the shaking process. Even the dirtiest wastewater samples can still be analyzed by this technique. This is not always the case with the more conventional extraction procedures where intractable emulsion formation may be a major problem. Extraction systems similar to Grob's showed a distinct tendency to form emulsions when tried in this laboratory.

Salt Effects--

Mieure (167) added sodium chloride to his samples to increase the ionic strength of the water layer which increased the extraction efficiency of his system. The sample shown was only for a sample/extractant ratio of 5:1. With this ratio, an increase in extraction efficiency from 93 to 98 per cent was observed. Our experiments showed no trend of increasing extraction efficiency with increasing ionic strength. We therefore, did not add salt to samples in our system for the purpose of increasing extraction efficiency.

Shaking Time--

Standard samples for the evaluation of shaking time on extraction efficiency were prepared by spiking organic free water and extracting for varying lengths of time. Times of 2-, 5-, 10-, 20-, and 30 minutes were examined. The resulting analyses indicated that equilibrium had been achieved within two minutes. Integrals for all the peaks in the sample were within the limits of precision of the technique relative to peak integrals of samples shaken for longer time periods. A shaking time of twenty-five minutes was arbitrarily selected because heavily polluted samples might take longer to equilibrate and because such a long shaking time can be used and still not be the limiting factor for the total analysis time.

Extractant/Water Ratios--

It is clear that the extractant/water ratio affects the extraction efficiency (E). The relationship between E and the extractant/water ratio is given by the following equation:

$$E = \frac{100D}{D + V_W/V_O}$$

Here E is the per cent of the VCO extracted, V_W is the volume of water used, and V_O is the volume of organic solvent used. D is the distribution coefficient which for dilute solutions of solutes (VCOs) should be a constant. Looking at published data for chloroform, Junk produces distribution coefficients from 40 to 53 (166). Data from this laboratory result in a D of 66; Mieure's data run from 49 to 114 (167). Although Grob and Grob did not analyze chloroform per se, their distribution coefficients generally run 1000 or greater. In describing their extraction system, Grob and Grob allude to the difficulty in achieving these remarkably high distribution coefficients (165), indicating that careful techniques had to be rigorously applied.

With the exception of Grob's data, LLE extraction efficiencies observed in this laboratory and by other workers show that increasing water/solvent ratios lead to decreasing

TABLE 29. RESPONSE FACTORS FOR VCO'S USING DIFFERENT ANALYTICAL TECHNIQUES

Compound	LLE	Modified[b] Bellar P/T	Direct[a] Aqueous Injection
$CHCl_3$	100	100	100
$C_2H_4Cl_2$	0.56	67	1
CCl_4	2000	67	1000
$CHBrCl_2$	500	50	200
$CHBr_2Cl$	333	40	100
$CHBr_3$	67	29	22

[a] Electron capture detection

[b] Coulson electrolytic conductivity detection

TABLE 30. PRECISION OF ANALYTICAL METHODS FOR ANALYSIS OF VCOs AT S/N 20-200 (PER CENT RELATIVE STANDARD DEVIATION)

Compound	LLE	Modified Bellar P/T	Direct Aqueous Injection
$CHCl_3$	1.9	1.8	1.5
$C_2H_4Cl_2$	3.8	2.2	---
CCl_4	2.1	5.2	---
$CHBrCl_2$	8.1	2.4	3.8
$CHBr_2Cl$	5.4	3.4	3.1
$CHBR_3$	4.7	6.6	---

TABLE 31. MINIMUM DETECTABLE LIMITS FOR THE ANALYSIS OF VCOs BY LLE, BELLAR D/T AND DAI METHODS (S/N = 2)

Compound	Detection Limit (µg/L in H_2O)		
	LLE	Modified Bellar	Direct Aq. Inj.
$CHCl_3$	0.2	0.2	1
$1,2\text{-}C_2H_4Cl_2$	36	0.3	90
CCl_4	0.01	0.3	0.1
$CHCl_2B_r$	0.04	0.4	0.5
$CHBr_2Cl$	0.06	0.5	1
$CHBr_2$	0.3	0.7	4.5

TABLE 32. EXTRACTION EFFICIENCIES OF VCOs BY THE PENTANE LLE METHOD

Compound	Extraction Efficiency (%)
$CHCl_3$	62
$1,2\text{-}C_2H_4Cl_2$	41
CCl_4	87
$CHBrCl_2$	69
$CHBr_2Cl$	72
$CHBr_3$	66

extraction efficiencies thus placing a practical limit on the concentration factor which can be achieved.

Matrix Effects--

Table 33 shows a comparison of the analytical techniques when applied to a "real world" sample (Denton, Texas tapwater). Clearly, in this situation the precision of the LLE method is better than that for the Bellar procedure (no precision values for DAI are available).

Most important is the discrepancy in the quantitative results between the DAI method and the other two methods for chloroform and bromodichloromethane. The DAI values are seen to be much higher than corresponding values for the other techniques. Nicholson (76) observed a similar trend with his DAI procedure. He contends that the higher values are due to haloform formation in the injector port of the gas chromatograph. Apparently, the heat catalyzes the chlorination and/or decomposition of chlorinated haloform precursors which increase the apparent haloform concentration. This is the primary objection to the method as an instantaneous VCO monitoring technique. These anomalous effects are not observed for the LLE procedure because the chloroform precursors apparently are not observed with the Bellar procedure because the precursors apparently are not purged from the water sample. Thus, these latter two methods more accurately reflect the instantaneous concentrations of the chlorocarbons.

Procedure--

It is difficult to compare quantitatively the procedural advantages which one technique has over another. However, analysis time is one parameter which can be accurately estimated. Table 34 shows a comparison of time of analysis for various numbers of samples. Clearly, the LLE method has a distinct time advantage over the Bellar procedure. The importance of this in a high sample volume survey program has been indicated above.

The LLE method has two other advantages over the Bellar procedure. Firstly, considerable special equipment is required for the Bellar process. The Bellar technique requires a special purge/trap apparatus which can either be purchased commercially for about $3,000, or can be built in-house, perhaps requiring several months of development time. At least some modifications are required in the injection port of a commercial chromatograph, and conventional chromatography via syringe injection is difficult while the Bellar apparatus is in place. Thus, the system is considerably less flexible than might be desired. Finally, an electrolytic conductivity detector is almost universally used (although other specialized detectors might potentially be used). For most laboratories, this means an additional $3,000 investment with the accompanying installation problems.

The extraction/concentration procedure of the LLE method is carried out in the sample bottle, eliminating the need for cleaning extraction equipment. Once extracted, the sample is immediately ready for chromatographic analysis. The LLE procedure uses a conventional electron capture gas chromatograph in the configuration supplied by the manufacturer. Thus, any laboratory equipped for pesticide analysis already has the necessary analytical instrumentation.

The other major procedural advantage of the LLE procedure is the technical expertise required by the analysts. The simplicity of the LLE procedure has resulted in competent analyses being performed by the least trained technicians in our laboratory. The Bellar method, on the other hand, has required highly skilled analysts in our laboratory who have had enough experience with this specific technique to understand the idiosyncrasies of the system. Generally, the Bellar method requires more sample handling, more hardware manipulation and leaves more room for "cockpit" errors.

There are two principal disadvantages of the LLE method as compared to the purge and trap procedure. One is the lower sensitivity of LLE method when GC/MS confirmation is attempted. The LLE method is sensitive primarily because of the use of the electron capture detector which detects picogram quantities of organohalides. If confirmation of the compounds is attempted by GC/MS, much higher concentrations are required since only a portion of the LLE solvent extract may be injected into the GC/MS system. On the other hand, virtually all of the VCOs in a sample go into the GC/MS system when the purge and trap procedure is used, thus, the detection limits are comparable to the values given in Table 31.

The LLE method as described above also cannot analyze complex mixtures of VCOs, particularly very volatile compounds such as vinyl chloride. This limitation may be minimized to some extent by the use of capillary columns and cryogenic temperature programming, but the purge and trap procedure is more convenient.

Recent Developments in the Analysis of VCO Compounds in Water

The LLE procedure described above was developed in 1975 and first reported at the "First Chemical Congress of the North American Continent" in Mexico City in December, 1975 (161). Most of the material contined in this section describes the results of early work on the development of the method. Subsequently, the method was used in a study of approximately twenty-five East Texas area water supplies (168) and has been

TABLE 33. ANALYSIS OF VCOs IN DENTON, TEXAS TAPWATER

Method	µg/L Halogen, as Cl*			
	$CHCl_3$	$CHBrCl_2$	$CHBr_2Cl$	$CHBr_3$
LLE	27.3 ± 0.3	25.7 ± 0.3	19.4 ± 0.4	4.24 ± 0.08
Modified Bellar	29.3 ± 2.2	32.5 ± 1.8	25.4 ± 1.0	n.d.
Direct Ag. Inj.	71.5	47.1	29.5	n.d.

n.d. not detected

TABLE 34. TIME TO COMPLETE MULTIPLE VCO ANALYSES BY LLE AND MODIFIED BELLAR METHODS (HRS)

Number of Samples in Set*	LLE	Bellar
1	1.6	3
2	2.5	4.5
4	4	6
8	6	13
16	10	25

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*Duplicate assays for each sample and QC samples included.

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used by numerous other groups for VCO analysis. In addition the USEPA has adopted a modified version of the Mieure method (167) as an acceptable THM method (160). Alternative techniques for the analysis of THMs and case histories of THM formation in water treatment plants have been compared recently by Brass (169).

Recently, a convenient LLE method has evolved which utilizes a 25 ml sample bottle from which five ml of water is removed and replaced by one ml of pentane (155). The 20 ml of water and one ml of pentane may be handshaken for one minute and analyzed directly by EC/GC. Recent optimization studies have shown that the extraction efficiencies in this system are higher than in the original method, and are relatively unaffected by changes in pH, salt content (up to moderate values), and the presence of up to 1% methanol. More significantly, recent studies have shown that n-pentane allows one to utilize the LLE method combined with glass or silica capillary GC columns for the analysis of a much broader range of VCOs. Lower detection limits of "purgeable organics" are higher on some cases than with the Bellar method (88) but quite acceptable for many applications. It is clear that capillary GC/LLE methods with either FID or EC detection are promising VCO analytical methods of the future.

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APPENDIX A

LIMITED CLUSTER SEARCH MASS SPECTROSCOPY

COMPUTER PROGRAM

The LCS computer program was originally written in BASIC computer language on the North Texas State University Hewlett-Packard 200 Timeshare System. This system could not access the Finnigan GC/MS data directly, but program debugging could be affected by manually inputting masses and intensities for single spectra.

Once the program was debugged, it was transferred to a second, more powerful Finnigan 6100 system located at the University of Texas Health Science Center, Houston, Texas. This system possessed the Finnigan BASIC interpretor which required a total of 16K of CPU core. The system could execute the BASIC program and directly access the previously acquired GC/MS data.

COMPUTER HARDWARE

The program is initiated by a pushbutton interrupt on the front panel of the computer system which activates the BASIC interpretor. Then the program instruction statements are ready to be entered manually via teletype or to be read in from magnetic tape storage. The GC/MS data must be stored on disk as spectra are sequentially retrieved from there. GC/MS data which has been previously stored on magnetic tape must be read onto the disk prior to program execution. Tabular LCS data is output after each spectrum is processed. Once all the spectra have been processed, a command can be entered which returns the system to the assembler-controlled mode deactivating the BASIC interpretor. This process automatically transfers any computed chromatogram or mass spectrum onto the cathode ray tube (CRT) display. In this case, the Limited Cluster Search chromatogram is transferred to the "GC OR UPPER" portion of the CRT. Using the assembler, the LCS data can be manipulated and plotted as though it were data which had been acquired in a conventional manner directly from the mass spectrometer.

DESCRIPTION OF THE PROGRAM

Appendix B is a listing of the LCS program. Figure A.1 shows a flow chart which highlights the important programming features of the Limited Cluster Search Program. These features are discussed below.

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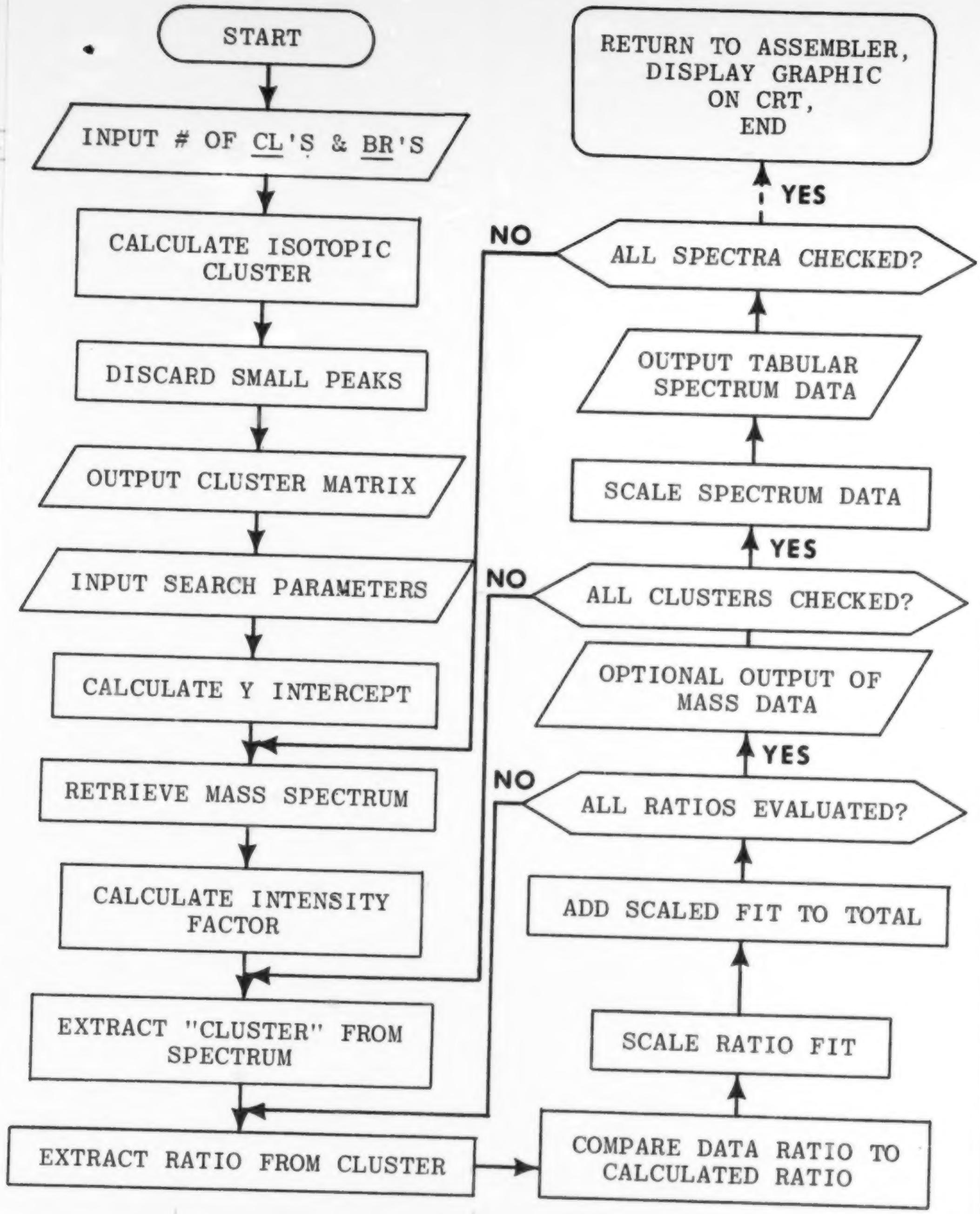
START
INPUT # OF CL'S & BR'S
CALCULATE ISOTOPIC CLUSTER
DISCARD SMALL PEAKS
OUTPUT CLUSTER MATRIX
INPUT SEARCH PARAMETERS
CALCULATE Y INTERCEPT
RETRIEVE MASS SPECTRUM
CALCULATE INTENSITY FACTOR
EXTRACT "CLUSTER" FROM SPECTRUM
EXTRACT RATIO FROM CLUSTER
COMPARE DATA RATIO TO CALCULATED RATIO
SCALE RATIO FIT
ADD SCALED FIT TO TOTAL
ALL RATIOS EVALUATED?
NO
YES
OPTIONAL OUTPUT OF MASS DATA
ALL CLUSTERS CHECKED?
NO
YES
SCALE SPECTRUM DATA
OUTPUT TABULAR SPECTRUM DATA
ALL SPECTRA CHECKED?
NO
YES
RETURN TO ASSEMBLER, DISPLAY GRAPHIC ON CRT, END

Figure A-1. Flowchart of limited cluster search program.

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Calculation of Isotope Cluster

Table A-1 shows the typical computer/programmer dialogy and data output of the program. The program parameters which are underlined are input by the programmer in response to computer querries, and the tabular data are output below. These data include the mass data output, which is optional, and the spectral data output.

The program begins by requesting the number of chlorine and bromine atoms for which the data will be searched. A total of 20 atoms in any combination may be input. Then the program calculates the relative peak heights for the cluster and outputs them as percentages with the base peak of the cluster being assigned 100 per cent.

The program uses two constants, 3.08664 for chlorine and 1.02041 for bromine, which represent the natural abundances for ^{35}Cl relative to ^{37}Cl and ^{79}Br relative to ^{81}Br, respectively. These constants were derived empirically in order to produce resulting isotope peak percentages which are in agreement with the U.S. Environmental Protection Agency mass spectral tables (193) to two decimal places. Either constant can be changed by altering the corresponding equality instruction statement in the program.

Isotope peaks which have an intensity less than seven per cent relative to the base peak of the cluster were discarded. This is done in an effort to avoid possible mismatches based on potentially not finding peaks of relatively low intensity.

Search Parameters

The following are input consecutively in response to computer queries: first and last masses; first and last spectra; baseline noise, percent; precision estimate; variation estimate. The first and last masses define the spread of masses to be examined in each spectrum. The same masses must be examined for all spectra in a given run, but not all masses which were acquired in a GC/MS run must be examined. The first mass selected was usually the mass of the smallest probable fragment for a given cluster. Thus, for a two-chlorine search, the smallest probable fragment was (CCl_2) at an m/e of 82.

The baseline noise parameter eliminated some computer calculations of trivial data. This was done by not comparing a data cluster to the calculated cluster if the intensity of the base peak for that data cluster was below the baseline noise per cent parameter relative to the base peak of the partial spectrum as defined by the first and lass mass search parameters. A baseline noise percentage of seven per cent was arbitrarily selected for all the data shown below.

TABLE A-1. LCS DIALOGUE

```
INPUT CL's THEN BR's
?2
?2

CL = 2  BR = 0
ISOTOPIC CLUSTER LISTING
A (1) = 100
A (2) = 64.7954
A (3) = 10.4961

INPUT LOWEST AND HIGHEST MASSES THEN-
FIRST AND LAST SPECTRA TO BE SEARCHED
?35
?160
?93
?93
BASELINE NOISE FILTER PER CENT
?7
INPUT PRECISION ESTIMATE
?2
INPUT VARIATION ESTIMATE
?-25

MASS = 35       CUMULATIVE FIT = 0
MASS = 39       CUMULATIVE FIT = 0.429949
MASS = 41       CUMULATIVE FIT = 0.859898
MASS = 46       CUMULATIVE FIT = 1.77292
MASS = 48       CUMULATIVE FIT = 2.68595
MASS = 53       CUMULATIVE FIT = 3.08933
MASS = 55       CUMULATIVE FIT = 3.49272
MASS = 69       CUMULATIVE FIT = 4.04368
MASS = 70       CUMULATIVE FIT = 4.89866
MASS = 71       CUMULATIVE FIT = 6.64601
MASS = 72       CUMULATIVE FIT = 7.95617
MASS = 73       CUMULATIVE FIT = 9.70351
MASS = 107      CUMULATIVE FIT = 12.1219
MASS = 109      CUMULATIVE FIT = 14.5402
MASS = 111      CUMULATIVE FIT = 16.9585
MASS = 142      CUMULATIVE FIT = 21.9585
MASS = 143      CUMULATIVE FIT = 22.3255
MASS = 144      CUMULATIVE FIT = 27.3255
MASS = 145      CUMULATIVE FIT = 27.6925
MASS = 146      CUMULATIVE FIT = 37.6925
MASS = 147      CUMULATIVE FIT = 38.4265
SPECTRUM=93     CUMULATIVE FIT = 38.4265  WEIGHTED FIT=17746.9
```

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The precision and variation estimation parameters relate to the decision process of determining whether a data cluster is that of a chlorine- or bromine-containing fragment. That process is described below.

DECISION PROCESS

A chart describing the flow of the GC/MS data as it is processed is shown in Figure A-2. Once the search parameters have been input, the program retrieves the first spectrum to be examined from the data file. Then, the first data cluster to be examined is extracted from that spectrum beginning at the first mass search parameter. The total number of peaks in the data cluster is equal to the total number of isotope peaks in the calculated cluster.

The comparison of the calculated cluster with the data cluster is a three step process. First, the difference between the two clusters is calculated. This calculation uses an arbitrary formula to derive the Z-value which is a measure of the ratio differences. The second step involves scaling the Z-value to produce the Cumulative Fit (CF). The scaling process accomplishes two objectives. It introduces two independent parameters into the calculations which allow the arbitrarily derived Z-value to be empirically optimized. And, it allows the establishment of a directly proportional relationship between the data and the numerical description of that data, the CF. The directly proportional relationship causes the baseline of the LCS chromatogram to occur at a Y-value of approximately zero and the peaks to extend in an upward direction from the baseline of the chromatogram. If the inversely proportional relationship which exists between the Z-value and the data ratios were used, the baseline would be located at an arbitrary positive value on the Y-axis and the peaks would extend in a downward negative direction. Thus, the scaling procedure is essentially an inversion of the Y-axis.

Once the CF has been calculated, other necessary factors are applied to produce the Weighted Fit (WF). This is the actual value that is plotted against the spectra numbers in an LCS chromatogram.

Z-value

The Z-value is the absolute numerical difference between the ratio of the peak heights of two adjacent peaks in the data cluster versus two corresponding peaks in the calculated cluster. It is formulated

$$Z = C_X/C_X + 2 - D_X/D_X + 2$$

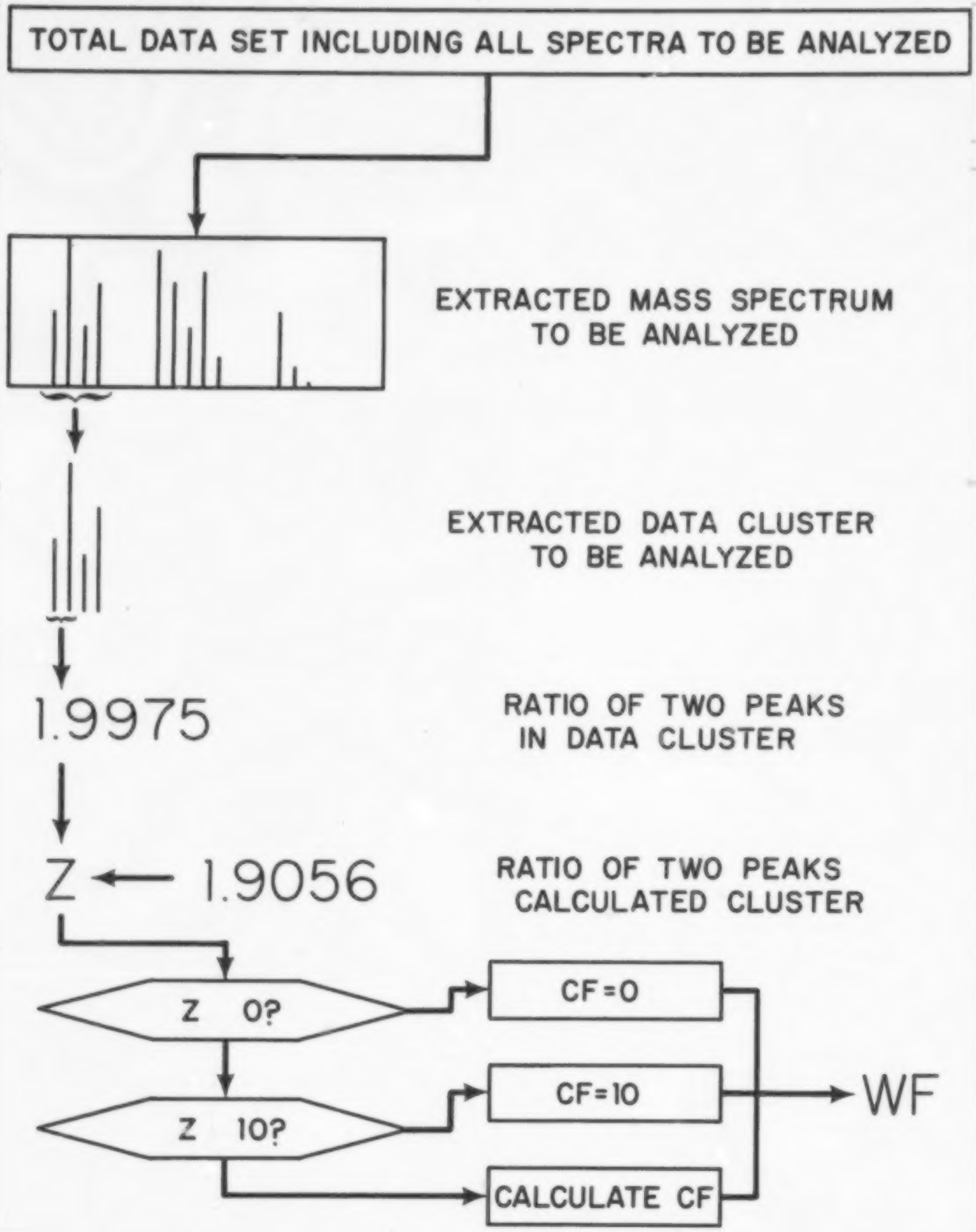
TOTAL DATA SET INCLUDING ALL SPECTRA TO BE ANALYZED
EXTRACTED MASS SPECTRUM TO BE ANALYZED
EXTRACTED DATA CLUSTER TO BE ANALYZED
1.9975
RATIO OF TWO PEAKS IN DATA CLUSTER
Z
1.9056
RATIO OF TWO PEAKS CALCULATED CLUSTER
Z 0?
CF=0
Z 10?
CF=10
CALCULATE CF
WF

Figure A-2. Flowchart of GC/MS data.

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where C is the ion intensity of a peak in the calculated cluster at mass X or X + 2, respectively, and D is the ion intensity of a peak in the data cluster at mass X or X + 2 respectively. As the relative data cluster peak heights approach the relative peak heights of the calculated cluster, the Z-value approaches zero indicating a high probability that the data cluster contains the same number of chlorines and/or bromines as the calculated cluster. Note that this formula compares the peak heights of only two adjacent peaks at a time. Therefore, n-1 Z-values will be generated for each total cluster examination where n is the number of peaks in the cluster. If the calculated cluster were derived from two chlorine atoms, for example, the cluster would contain three isotope peaks, and two Z-values would be produced. This is important in analyzing raw data as shown below.

An example of the relationship between the Z-value and a data peak ratio is shown in Figure A-3. This example refers to the first Z-value generated for a dichlorinated isotopic cluster. In such a calculated cluster, the relative peak heights of the X and X + 2 peaks are 100 per cent and 64.7954 per cent, respectively. The X-axis in Fig. A-3 shows the percent deviation of the X + 2 peak in the data cluster from that perfect relative peak height as defined in the calculated cluster (64.7964 per cent). This can be formulated as

$$Z = 100.0/64.7954 - 100/(64.7954 \pm \% \text{ deviation}).$$

Thus, if the mass spectrometer recorded the D_{X+2} peak in the data cluster with a relative intensity of 62.7954%, the per cent deviation would be -2.0, and the corresponding Z-value would be 0.049.

Cumulative Fit

The scaling procedure which converts the Z-value to the CF, is itself a three step process as indicated in Figure A-2 Initially, the CF is calculated according the formula

$$CF = VZ + C$$

where V is the variation estimate parameter, and C is a dependent variable which is a function of both the precision estimate and the variation estimate parameters. As indicated in Fig. A-4, the CF has a range of 0.0 to 10.0. The 10.0 CF value indicates a "perfect fit" between the data cluster and the calculated cluster. Note that this value corresponds to some Z-value which is always greater than zero and is defined by the precision estimate as described below. The 0.0 CF value indicates the fit between the data cluster ratio and the calculated cluster ratio are so poor that there is no probability that the data cluster could represent an ion fragment which contains the proper number of chlorines and/or bromines. Fits can be worse than

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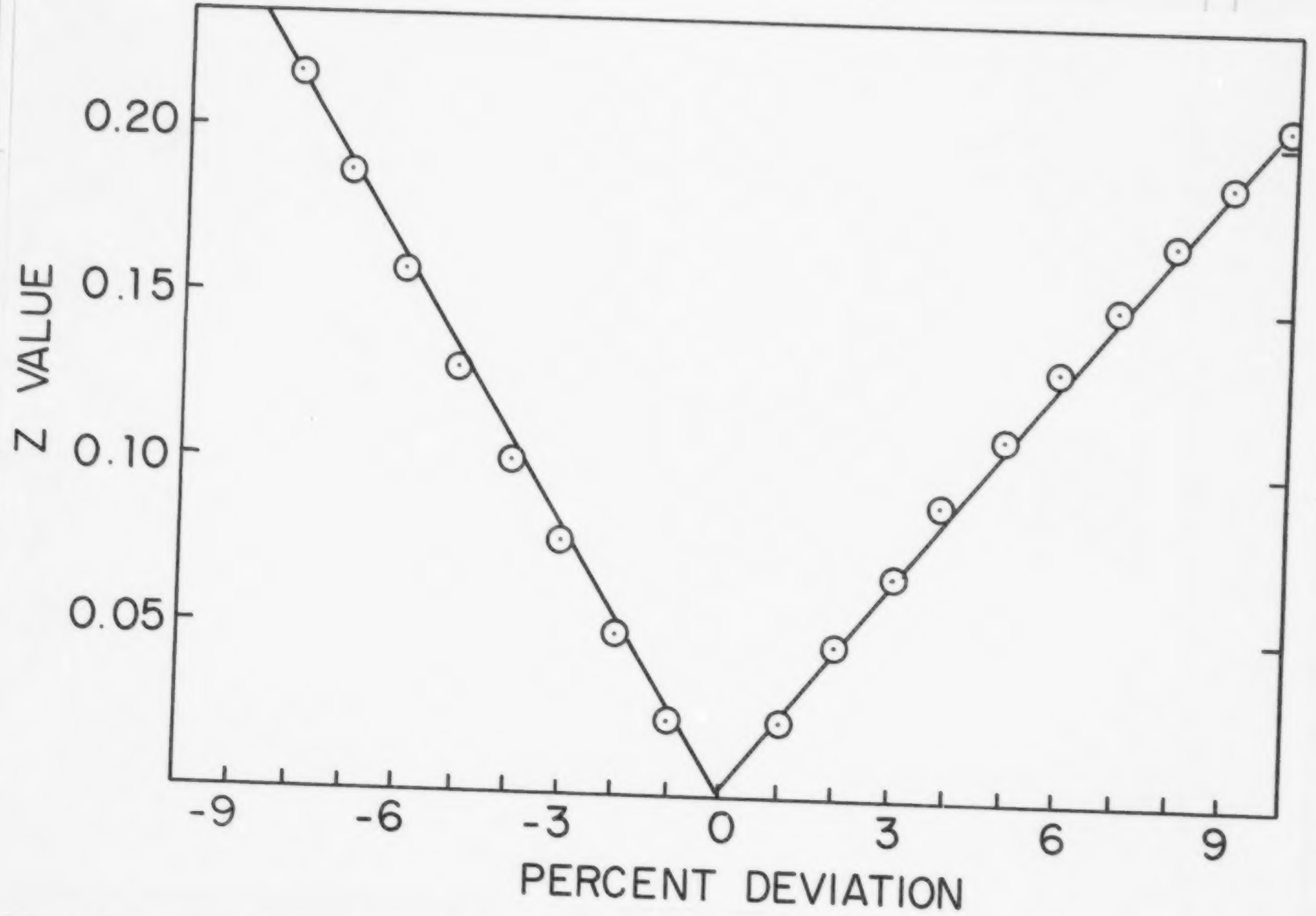

Figure A-3. Relationship between Z-value and percent deviation of the M+2 peak in a dichlorinated isotopic cluster.

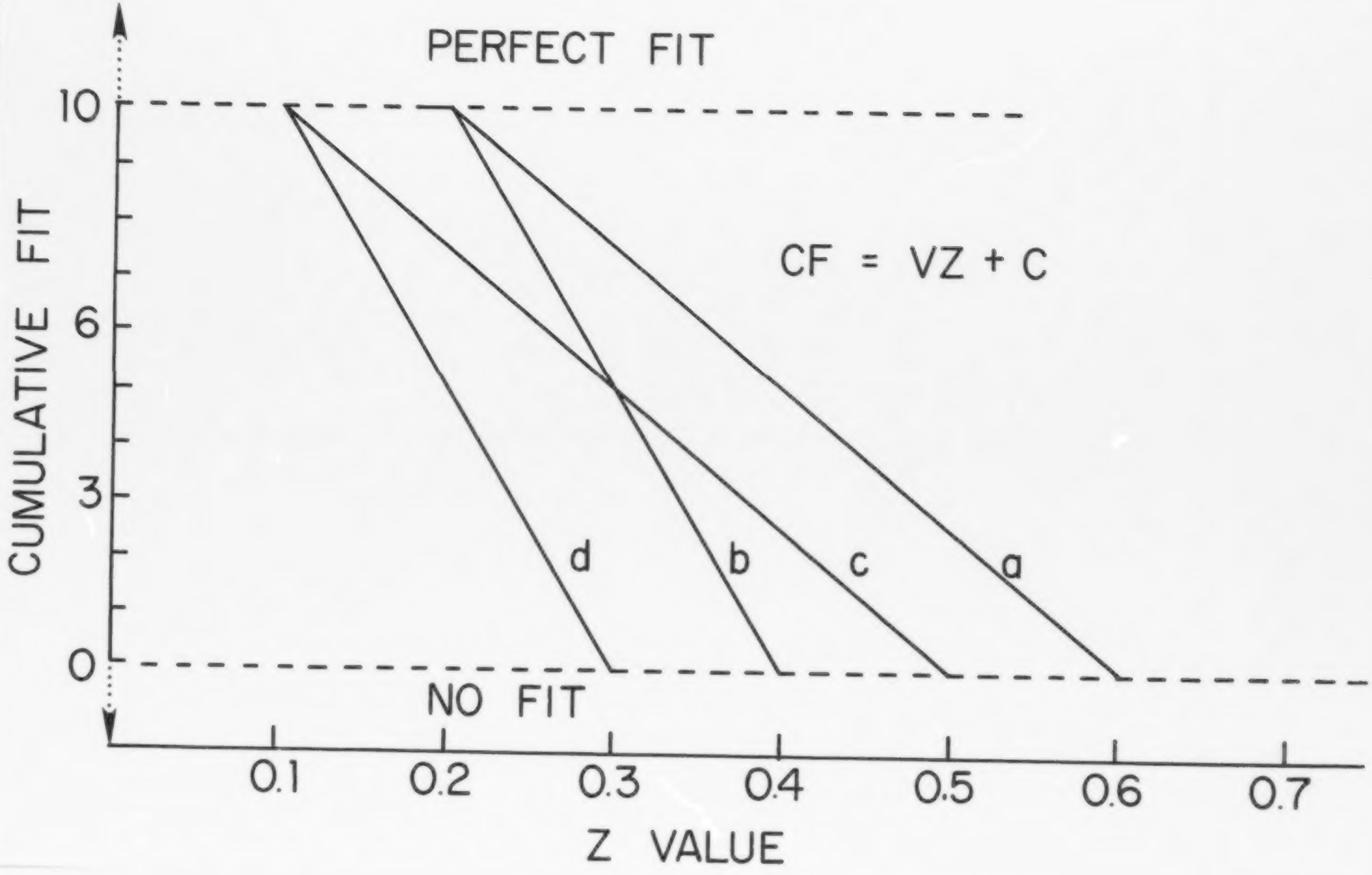

Figure A-4. Relationship between cumulative fit and Z-value.

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this and generate negative CF's. However, since the probability of a fit can be no worse than zero, the lowest rational CF value is 0.0, and negative values are adjusted to that value.

The Precision Estimate Parameter

The precision estimate parameter reflects the spread of values (precision) which the mass spectrometer produces when the same species is analyzed repeatedly. This spread usually produces a Gaussian-shaped curve when the intensity values are plotted against the frequency of occurrence of each value (107).

Within this spread no reliable distinction can be made between a match and a mismatch. Therefore, the precision estimate defines the maximum Z-value below which improvements in fits cannot be distinguished. CF values which are greater than the 10.0 perfect fit are automatically reset to the perfect fit value.

The Variation Estimate Parameter

The variation estimate parameter (V) adjusts for the other primary contribution to the variation of the relative data peak heights from the theoretical values. This deviation has several sources. They all result in the unequal spurious contribution of ion intensity to peaks in the data cluster. These unequal contributions cause distortions in the Z-values, and consequently in the CF's. Instrument background and chromatographic column bleed are primary sources of this effect. Contributions from nonhalogen atoms can also cause distortions. Atoms such as oxygen, silicon, sulfur, and even hydrogen can contribute to the distortion of X + 2 peak heights due to significant contributions of the X + 1 and X + 2 isotopes of these atoms. This effect is more pronounced in fragments which occur at higher m/e values. Therefore, a provision was made in the program which allows for a partial fit that indicates some, though less than certain probability that the data cluster contains the appropriate number of chlorines and/or bromines. The partial fit region of Fig. A-4 shows the line which relates to CF partial fits to the corresponding Z-values. Note that the variation estimate parameter controls that relationship since it is the slope of that line. As the variation estimate parameter becomes a larger negative number, the decision process approaches a "YES/NO" system. In such a system, the decision depends only on the value of the precision estimate; below this value a perfect fit is indicated, and above this value a perfect miss, no probability of a fit, is indicated. Such a system has a high risk of misinterpreting data which happens to fall close to the decision boundary. Examples of such data will be seen later.

Both the precision estimate and the variation estimate parameters were empirically optimized using the HALSTI data set.

The optimum combination was then used to process the CALCLI data set (see Section 5 for results).

OTHER SCALING FACTORS

Three other scaling factors are required in this program including the halogen number factor, the cluster intensity factor, and the spectral intensity factor.

The Halogen Number Factor

For each complete cluster analysis, n-1 CF's are generated where n is the number of peaks in the calculated cluster. Since the total CF for a perfect match between the calculated cluster and the data cluster has been defined as 10.0, the CF for each ratio within a given cluster must be divided by (n-1), the halogen number factor, in order that each CF contributes the appropriate fractional contribution to the total CF for the cluster.

If a calculated cluster contains three peaks, as it would in an LCS for two chlorines, two CF's would be generated for each cluster analysis. Therefore, each CF must contribute 5.0, 10.0/(3-1), to the total CF for the cluster. This effect is important in comparing two different LCS's of the same data set. Using this factor, a perfect fit of an LCS for two chlorines would generate the same CF as would a perfect fit for three chlorines. This is indicated in Fig. A-5 which shows raw spectra (no background subtracted) from the HALSTI data set. The dichlorobenzene spectrum was searched for ion fragments containing two chlorines. A perfect fit for one dichlorinated ion cluster was detected as indicated by the CF of 10.0. The trichlorobenzene spectrum was searched for ion fragments containing three chlorines. The CF of 9.6 indicates a partial fit which is almost equal to one trichlorinated ion fragment. The 0.4 unit error in the CF is due to the recorded ion intensity of the X + 2 peak in the data cluster which begins at m/e 180 (the molecular ion cluster). The relative value for the peak was 92.8% versus 97.2% for the corresponding peak in the calculated cluster. This -4.4% deviation in the relative peak height causes the first two Z-values for the cluster to be outside the precision limits of a perfect fit. Therefore, the CF's for those Z-values will be determined in the partial fit sector of the decision process, and the values of those CF's will be less than the perfect fit of 3.3333, 10.0 (4-1). Note that if the decision process had been a "YES/NO" system, the total CF for the cluster would have been only 3.3333 because the first two CF's would have been zero.

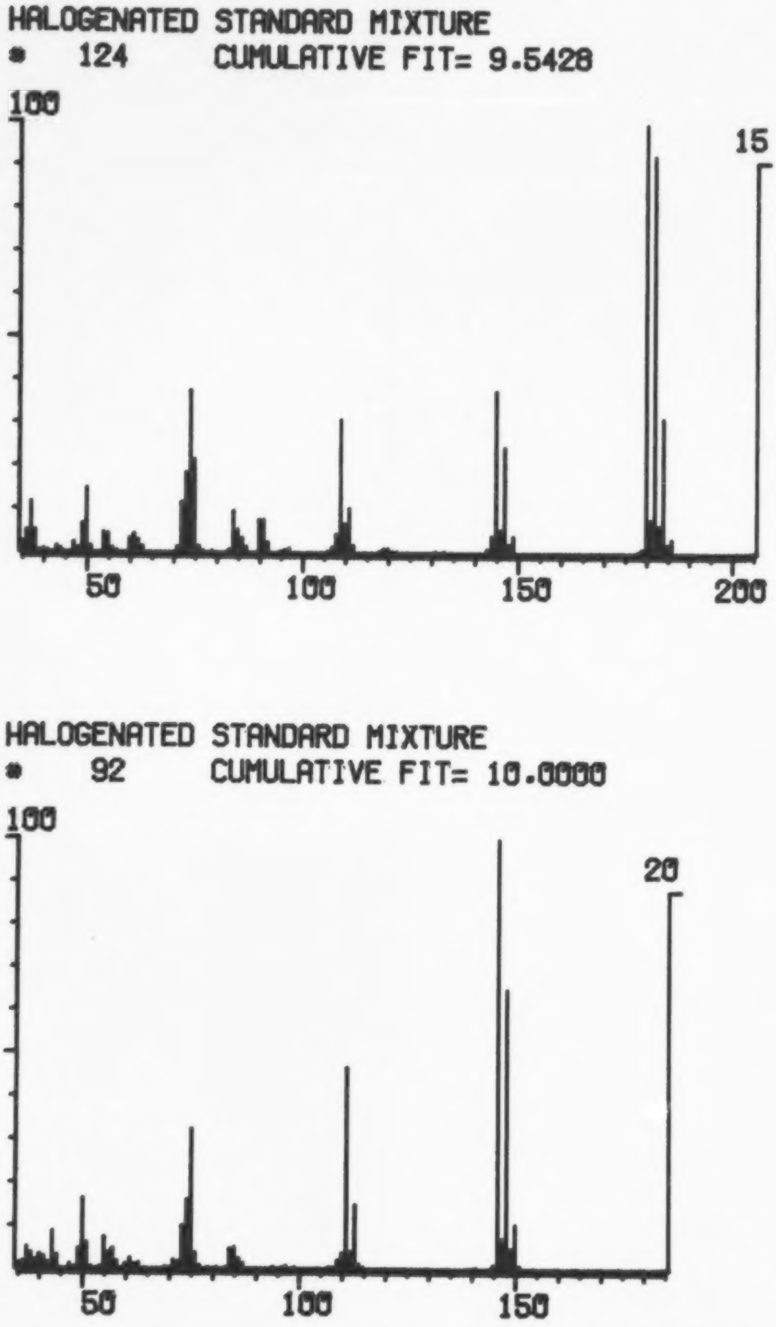

Figure A-5. Mass spectra (no background subtracted) of dichlorobenzene and trichlorobenzene (HALSTI data set).

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The Cluster Intensity Factor

Because of the ability of chlorine and bromine to stabilize a positive charge, ion fragments containing these atoms tend to produce mass spectral clusters with high relative intensities. To take advantage of this observation, the cluster intensity factor was developed. This factor is simply the base peak of the data cluster divided by the base peak of the partial mass spectrum as defined by the first and last mass input parameters. All newly generated CF's are multiplied by this factor before they are added to the total CF. This de-emphasizes good fit for clusters containing relatively small peaks. Such clusters often do not contain halogen atoms at all, but rather contain noise-like inconsequential data which coincidentally happens to resemble the calculated cluster. The factor tends to emphasize a good fit for a cluster containing relatively high intensity peaks which is often an accurate indication that the ion fragment does indeed contain the proper number of chlorine and/or bromine atoms. The trichlorobenzene spectrum shown in Figure 8 contains a data cluster which was generated by a dichlorinated ion fragment beginning at m/e 145, (m-35)+. The fit when the spectrum was searched for two chlorines, for the cluster beginning at m/e 145 was perfect, but the base peak of that cluster was only about 38% relative to the base peak of the partial spectrum at m/e 180. Therefore, the total CF was 3.8, 10.0 times 0.38.

The Spectral Intensity Factor

The mass spectrometer scans the GC effluent once every four seconds. An analyte requires about twenty to forty seconds to elute from the column. Thus, about five to ten scans are made of that analyte as it passes into the mass spectrometer. Although all of these spectra are practically identical, the TIC of that analyte peak has roughly a Gaussian shape. This is because the RGC reflects the absolute intensity of the ion fragments in the mass spectrum as opposed to the relative intensities of the ions. The absolute intensities are proportional to the instantaneous concentration of the analyte in the spectrometer as the scan is being made. The LCS program, however, is a function of the relative ion intensities. Therefore, the CF's of all the spectra acquired during the elution of a GC peak tend to have the same value regardless of the absolute intensity of the ion fragments and the LCS analyte peak shape tends to be rectangular in shape rather than Gaussian.

To achieve the desired Gaussian peak shape, the total CF for the LCS of the spectrum is multiplied by the spectral intensity factor. This factor is simply 0.01 times the total ion current for the partial spectrum as defined by the first and last mass input parameters. The 0.01 multiplicative factor is necessary so that the product of the CF times the spectral intensity factor will be about the same order of magnitude as the TIC.

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This is important to the computer internally in plotting the LCS data. The product of the CF times the spectral intensity factor is called the "Weighted Fit" (WF). The WF is the actual value which is plotted along the ordinate axis in the LCS chromatogram.

Fig. A-6 shows the relationship between the Total Ionization Chromatogram, the Cumulative Fit and the Weighted Fit which is the Limited Cluster Search chromatogram. The data set is being searched for dichlorinated compounds. The TIC indicates all of the compounds which eluted from the GC-column including chlorination and nonchlorinated compounds alike. The LCS chromatogram indicates only those peaks which contain dichlorinated ion fragments. Note the square shape of the CF chromatogram "peak" of the dichlorobenzene peak (spectrum numbers 89 through 99). This is also observed to some extent for the trichlorobenzene peak (spectrum numbers 122 through 127) and the hexachlorobutadiene peak (spectrum numbers 147 through 157).

The "noise" in the CF chromatogram between spectra 100 and 120 is caused by traces of dichlorobenzene which are adsorbed to the ion source housing of the mass spectrometer. As it desorbs, a dichlorobenzene spectrum of low absolute intensity is produced. However, since no other chromatographic peak elutes at that time, and since the instrument background and chromatographic column bleed are both low, the weak dichlorobenzene spectrum still accounts for the most significant ions in the scans. Therefore, the CF had a high value although "noisy" because the ion intensities were close to the detection limit of the instrument. When this factor is multiplied by the spectral intensity factor, the resulting WF is quite low as observed in spectrum numbers 100 through 120. This same phenomenon is observed at spectra numbers 130 through 138. Note that when a nonchlorinated analyte, n-tetradecane, begins to elute from the chromatographic column at spectrum number 137, the residual adsorbed trichlorobenzene becomes insignificant and the CF drops to 0. Thus, although the spectral intensity factor is high, the LCS response is zero because the CF is zero.

HALSTI: EMPIRICAL OPTIMIZATION OF THE SEARCH PARAMETERS

A detailed preliminary study of the relationship between the precision estimate parameter and the variation estimate parameter was made in an effort to select starting values for the subsequent empirical optimization study. Spectra from the apices of two GC peaks in the HALSTI data set were used for the preliminary study. The peaks were bromoform, spectrum number 46, and dichlorobenzene, spectrum number 92. Spectra of the raw data (no background subtracted) are shown in Figure A-7. Both spectra were subjected to a series of LCS searches for dichlorinated ion fragments. The bromoform spectrum shows a dibrominated ion fragment, $(M-Br)^+$, of high relative intensity whose data cluster begins at m/e 171. The X + 2 and X + 4 peaks of this

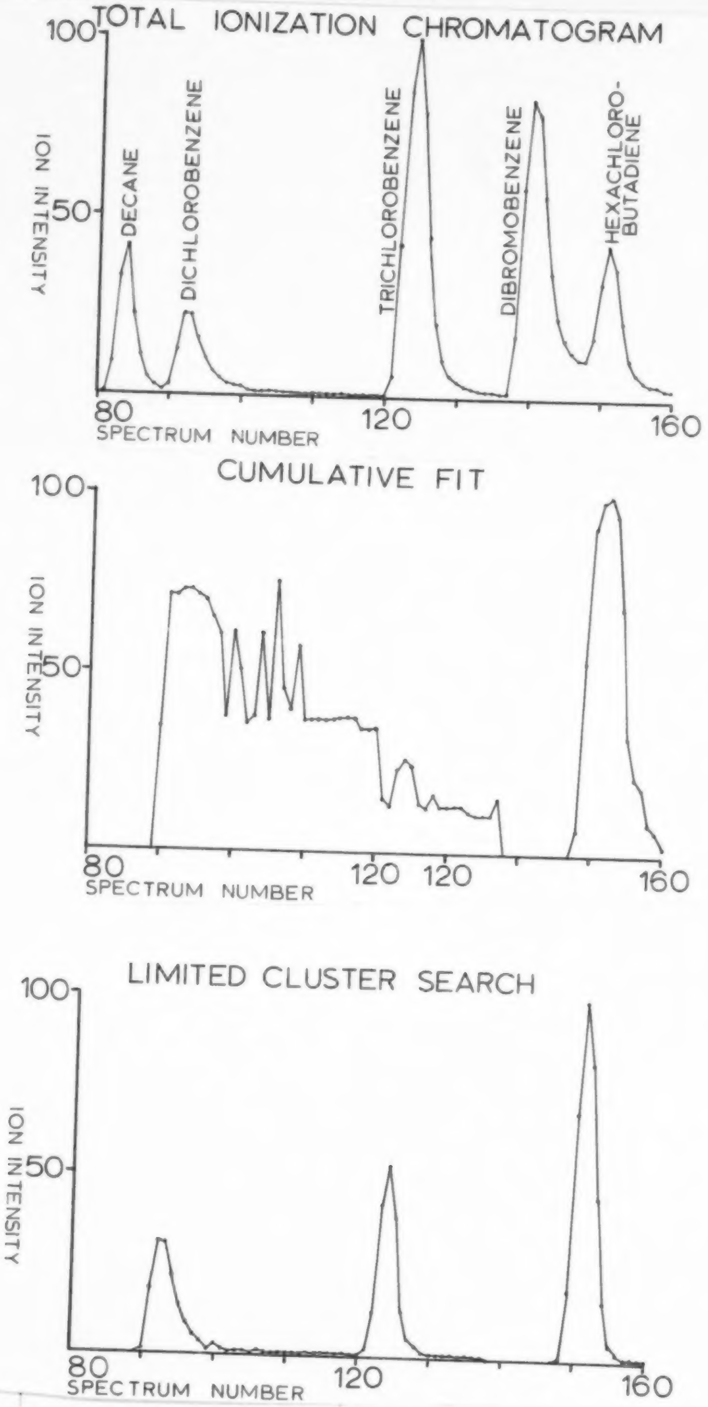

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Figure A-6. TIC and limited cluster search chromatograms for mixture of halogenated compounds.

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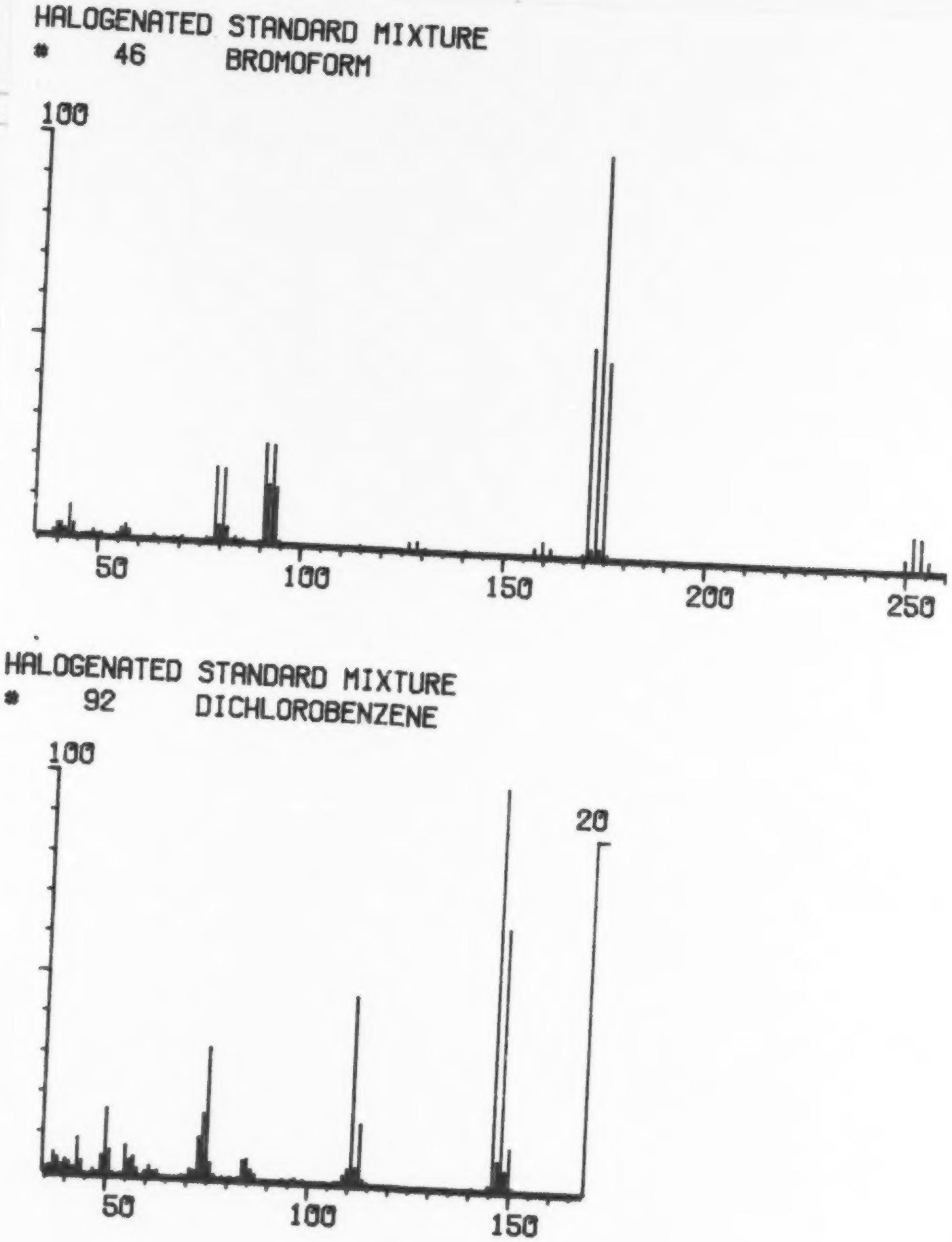

Figure A-7. Mass spectra of dichlorobenzene and bromoform (no background subtracted).

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cluster have a similar relative height relationship as that of the X and X + 2 peaks in the calculated cluster. Because of this, bromoform can show a partial fit for the dichlorinated LCS at m/e 173. The value of the CF will depend on the values of the precision estimate and variation estimate parameters. Such a mismatch, as indicated by a bromoform peak in the LCS chromatogram, should be considered an interference. The dichlorobenzene mass spectrum shows the expected intense dichlorinated data cluster beginning at m/e 146, the M^+ cluster.

Figure A-8 shows the data from this preliminary study. The study comprised three series of comparative LCS's. Each graph shows the Weighted Fit versus the Precision Estimate. A single variation estimate parameter is used in each graph. In all three cases, for low values of the precision estimate parameter where the fit requirements are strenuous, the bromoform spectrum shows a WF of zero. This indicates zero probability of the spectrum containing a dichlorinated data cluster. The dichlorobenzene spectrum shows a constant positive WF indicating a perfect match. As the precision input parameter is increased, the fit requirements are less strenuous, and the computer program begins to incorrectly indicate some probability that the bromoform spectrum contains a dichlorinated data cluster. As the value of the precision estimate parameter is increased further, the value of the WF for the dichlorobenzene spectrum also begins to increase due to the interfering mismatch of the data cluster beginning at m/e 111, an isotopic cluster produced by a monochlorinated ion fragment.

Comparison of the three graphs shows the effect of the variation estimate parameter on the decision process. With a variation estimate parameter value of -25, the precision estimate parameter must be set at a low value (less than 0.01) in order to avoid possible mismatches for the bromoform spectrum. However, for higher precision estimate parameter values at this variation estimate parameter value, the effect of the mismatch is diminished because the system is generally less sensitive to mismatches as indicated by the relatively gradual slope of the line showing the increasing bromoform interference. With a variation estimate parameter of -75, the system is extremely sensitive to mismatches, as indicated by the steep slope of the increasing bromoform interference, but the latitude of the precision parameter before a mismatch is detected is much greater. The graph showing the data with a variation estimate parameter value of -50 appears to be a good compromise between sensitivity and precision estimate latitude. This value was therefore selected as the initial variation estimate parameter. It was compared to the value of -25 in an effort to avoid the extreme sensitivity observed with the high variation estimate parameter value.

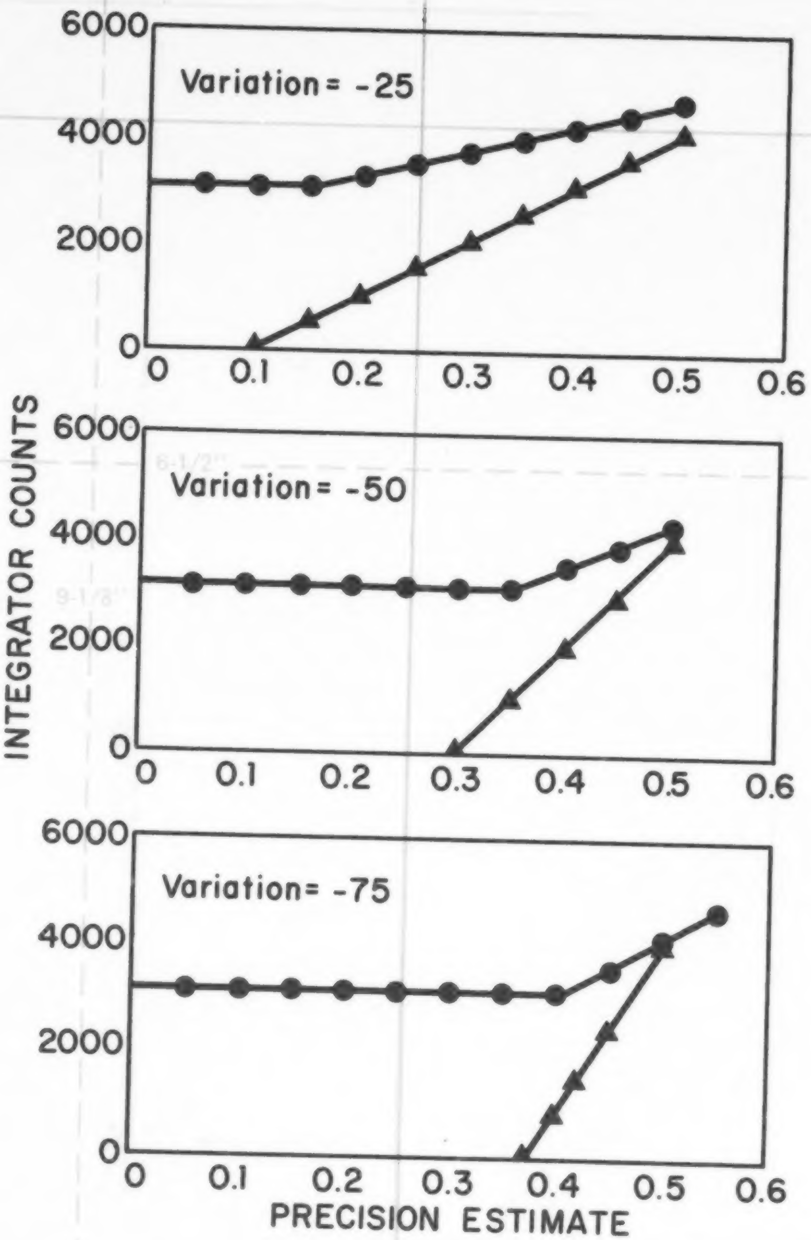

Figure A-8. Weighted fit vs. precision estimate for three values of the variation estimate parameter. ● dichlorobenzene; ▲ bromoform.

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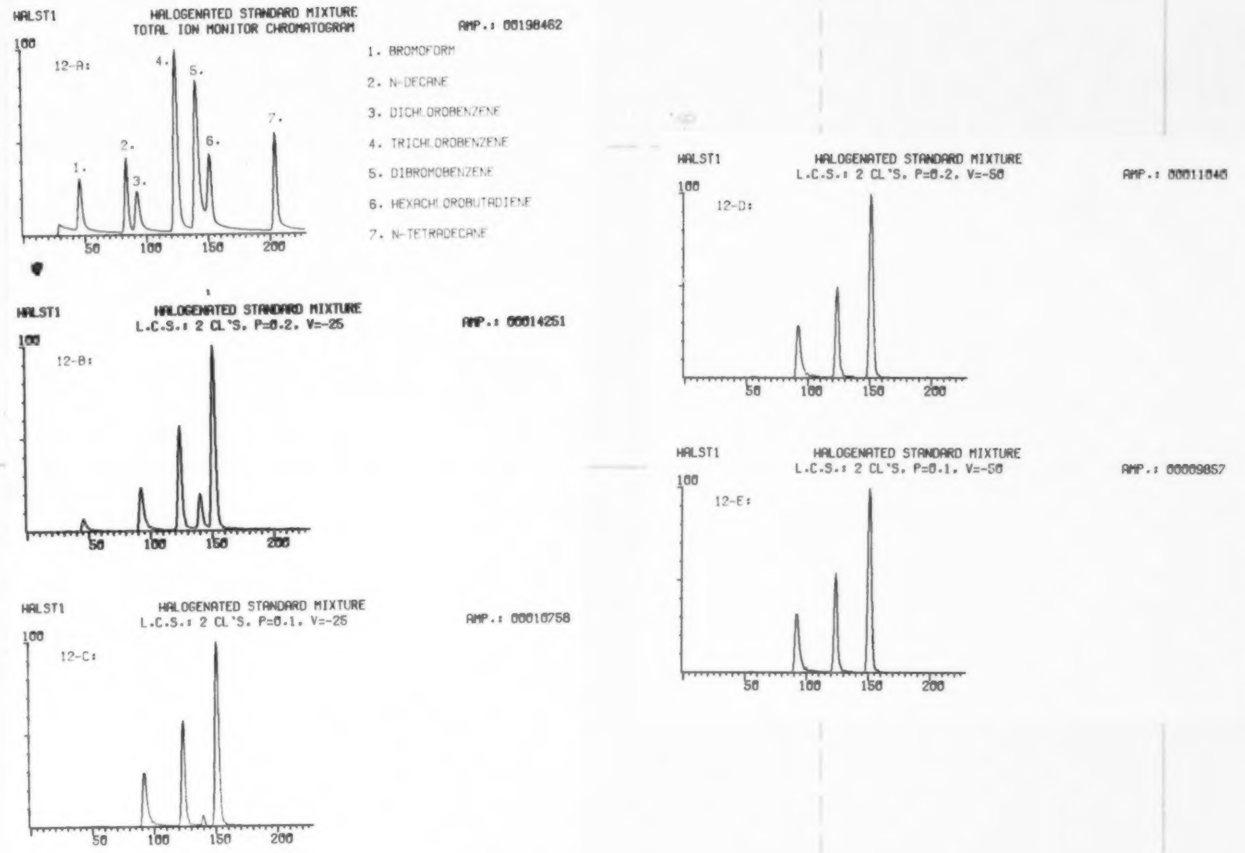

Figure A-9. Limited cluster search chromatograms for HALSTI data set with various precision and variation estimate parameters.

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The initial precision estimate parameters were selected based on a second preliminary study which was an investigation of the precision of the mass spectrometer. A series of ten 100 nanogram injections of dichlorobenzene were made. The per cent deviation of the relative height of the (M + 2)$^+$ ion at m/e 148 was compared against the 64.7954% relative height of the X + 2 peak in the calculated cluster. The data values ranged from 62.11% to 65.69% corresponding to deviations of -2.69% and 0.89%, respectively. The Z-values for these deviations were 0.067 and 0.021 (note that both positive and negative per cent deviations produce positive Z-values.) Since this sample was analyzed under ideal conditions, the minimum Z-value selected for use as the initial precision estimate parameter was 0.1, approximately twice the average of the Z-value deviations observed in the preliminary study. A value of 0.2, twice the minimum value, was used for comparison.

Figure A-9 shows the TIC and the *LCS's* for the entire HALSTI data set. The four possible combinations of the two precision estimate parameters and the two variation estimate parameters were used. Chromatograms 12-B and 12-C show the *LCS's* using a variation estimate parameter of -25. No interference is observed in either chromatogram from the normal alkanes in the data set. Some interference is observed from the dibrominated compounds demonstrating the lack of adequate discriminating ability associated with the variation estimate parameter value. However, the decreased peak heights of the interfering analytes relative to the height of the peaks which contain dichlorinated data clusters indicate the decreased sensitivity of the system to the interferences.

Chromatograms 12-D and 12-E show the *LCS's* using a variation estimate parameter of -50. In these chromatograms, no interference is observed from the normal alkanes, and the interference from the dibrominated analytes is also eliminated. Although the two chromatograms appear to be identical, subtle differences do exist as indicated by the differences in the amplitude values. These values are the *WF's* of the apical spectra of the tallest analyte peak in the chromatogram, in this case hexachlorobutadiene. Figure A-10 shows the raw spectrum, and Table A-2 shows the CF data for that spectrum. The CF's for m/e's 82, 94, 106, and 118 represent the proper recognition of clusters formed by dichlorinated ion fragments. The CF for m/e 143 is actually a mismatch of the X + 2, X + 4, and X + 6 peaks in a trichlorinated data cluster which begins at m/e 141. The CF for m/e 155 is a mismatch of the X + 4, X + 6, and X + 8 peaks in the data cluster generated from a pentachlorinated ion fragment beginning at m/e 153. The CF's at m/e 225 and 227 are mismatches of various combinations of peaks from a data cluster of pentachlorinated ion fragment beginning at m/e 223. Note that the total CF's for the clusters which are produced from dichlorinated ion

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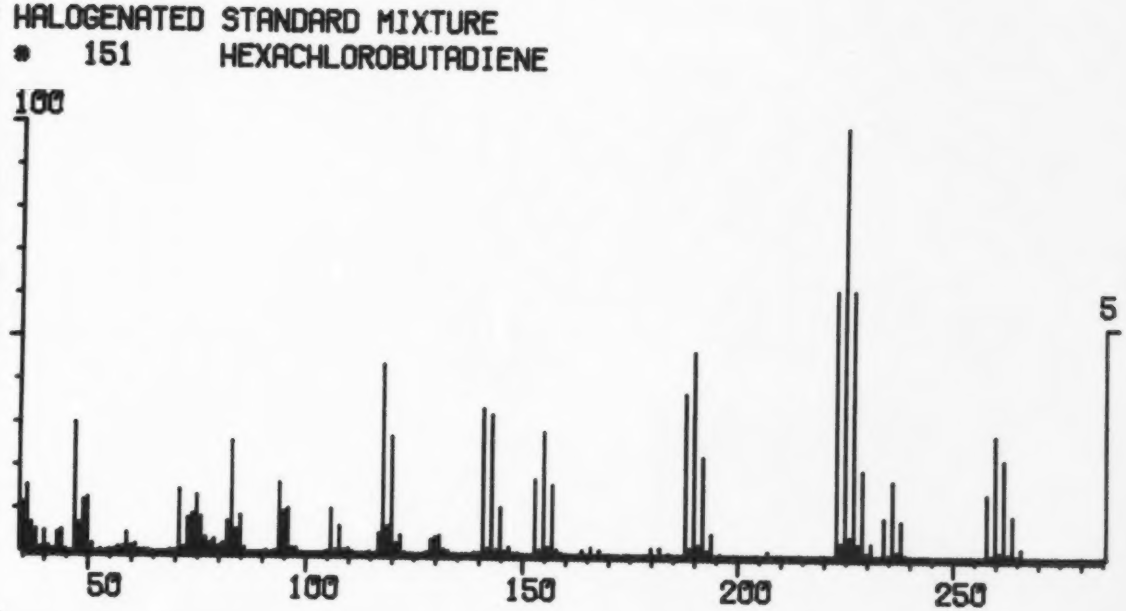

Figure A-10. Mass spectrum of hexachlorobutadiene (no background subtracted).

fragments are approximately the same for the two different precision estimates, 6.13 versus 6.04. However, the higher CF for the 0.2 precision estimate parameter indicates that it will exhibit a higher sensitivity to mismatches than will the 0.1 value. Therefore, values of 0.1 for the precision estimate and -50 for the variation estimate were selected as optimum values for the decision process. These values were then used for the analysis of the CALCLI data set (Section 5).

TABLE A-2. CUMULATIVE FIT VALUES FOR HALSTI DATA SET (cf. FIGURE A-10) HEXACHLOROBUTADIENE

	Precision Estimate Value	
m/e	0.2	0.1
82	0.38	0.28
94	1.21	1.11
106	1.73	1.64
118	6.13	6.04
143	6.79	0
155	8.23	6.80
225	13.23	11.80
227	16.35	14.60

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APPENDIX B

PROGRAM: LIMITED CLUSTER SEARCH (LCS)

```
  1  DIM M(1000),G(1000)
  5  FILES Z7
 10  DIM A(21),B(21)
 20  DIM TS(50)
 30  PRINT "INPUT CL'S THEN BR'S"
 40  INPUT N,L
 45  LET T=-1
 50  MAT A=ZER
 60  MAT B=ZER
 70  MAT G=ZER
 80  MAT M=ZER
 90  REM **********  CALCULATE CHLORINE CLUSTER ***************
100  IF N=O THEN 220
110  LET R=3.08664
120  LET A(1)=R
130  LET A(2)=1
140  IF N=1 THEN 220
150  FOR W=2 TO N
160  FOR I=1 TO W
170  LET A(W-I+2)=A(W-I+2)*R+A(W-I+1)
180  NEXT I
190  LET A(1)=A(1)*R
200  NEXT W
210  RE1 **********  CALCULATE BROMINE CLUSTER  ***************
220  IF L=O THEN 400
230  LET X=L
240  LET R=1.02041
250  IF A(1)>O THEN 300
260  LET A(1)=R
270  LET A(2)=1
280  LET X=L-1
290  IF L=1 THEN 400
300  FOR W=1 TO X
310  FOR I=1 TO 19
320  LET A(21-I)=A(20-I)
330  NEXT I
340  LET A(1)=O
350  FOR I=1 TO 19
360  LET A(I)=A(I+1)*R+A(I)
370  NEXT I
380  NEXT W
```

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```
390  REM ********** BASE PEAK:  ISOTOPIC CLUSTER ***********
400  LET W=O
410  FOR I=1 TO 20
415  LET W1=A(I)
420  IF W1<W THEN 440
430  LET W=W1
440  NEXT I
450  REM ********** CLUSTER ADJUSTMENT-CHUNK SMALL PEAKS ***
460  LET Q=O
470  FOR P=1 TO 20
480  IF A(P)>.07*W THEN 560
490  IF A(P)=0 THEN 590
500  FOR R=P TO 20
510  LET A(R)=A(R+1)
520  NEXT R
530  LET Q=Q+1
540  IF P>W THEN 560
550  LET P=P-1
560  NEXT P
580  REM **********  PRINT ISOTOPIC CLUSTER  ***************
590  PRINT
600  PRINT
610  PRINT "CL=";N;"BR=";L
620  PRINT "ISOTOPIC CLUSTER LISTING"
630  IF A(W)=O THEN 680
640  FOR I=1 TO 21
650  IF A(I)=O THEN 670
660  PRINT "A(";I;")=";A(I)/A(W)*100
670  NEXT I
680  PRINT
690  PRINT
700  LET N=N+L-Q
710  REM **********  INPUT SEARCH PARAMETERS ***************
720  PRINT "INPUT LOWEST AND HIGHEST MASSES THEN-"
730  PRINT "FIRST AND LAST SPECTRA TO BE SEARCHED"
750  INPUT L,H,C,F
760  REM **********  INPUT DATA BASELINE NOISE FILTERING ***
770  PRINT "BASELINE NOISE FILTERING, PERCENT"
780  INPUT D
910  LET D=D/100
920  REM ********** INPUT PRECIS. AND VARIATION ESTIMATES **
930  PRINT "INPUT PRECISION ESTIMATE
940  INPUT X1
950  PRINT "INPUT VARIATION ESTIMATE"
960  INPUT M1
970  REM **********  CALCULATE Y INTERCEPT  ****************
980  LET B1=10-(M1*X1)
990  REM **********  SPECTRUM SELECTION LOOP  **************
1000 FOR I=C TO F
1010 DREAD #1,I,M
1020 REM ********** CALCULATE SPECTRUM INTENSITY FACTOR ****
1030 REM *************** AND BASE PEAK, SPECTRUM ***********
```

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```
1040  LET R=0
1050  LET E=M(L)
1060  FOR J=L TO H
1070  LET E2=M(J)
1080  IF E2>0 THEN 1100
1090  LET M(J)=.01
1100  LET E=E2
1120  LET R=R+E2
1130  NEXT J
1140  LET R=R*.01
1150  LET G(I)=0
1160  REM ********** CLUSTER SELECTION LOOP *****************
1170  FOR J=L TO H-2*N
1180  REM ********** COPY DATA CLUSTER INTO MATRIX B ********
1190  REM ********** CALCULATE BASE PEAK FOR CLUSTER ********
1200  LET B3=0
1210  FOR P=1 TO N+1
1220  LET B(P)=M(J+(P-1)*2)
1230  LET B2=B(P)
1240  IF B3>B2 THEN 1260
1250  LET B3=B2
1260  NEXT P
1270  REM ********** ASSIGN CLUSTER HEIGHT SCALING FACTOR ***
1280  LET X=B3/E
1290  REM ********** TEST: CLUSTER EXCEEDS NOISE CUTOFF *****
1300  IF B3<D*E THEN 1450
1310  REM ********** RATIO SELECTION LOOP *******************
1320  FOR P=1 TO N
1330  REM ********** CALC. DIFF. OF DATA & THEOR. RATIOS *****
1340  LET Z=ABS(A(P)/A(P+1)-B(P)/B(P+1))
1350  REM ********** FIT WORSE THAN ZERO *******************
1360  IF B1<-M1*Z THEN 1430
1370  REM ********** FIT BETTER THAN PRECISION **************
1380  IF Z>X1 THEN 1410
1390  LET G(I)=G(I)+10*X/N
1400  GO TO 1430
1410  REM ********** ZERO < FIT < TEN **********************
1420  LET G(I)=G(I)+(M1*Z+B1)*X/N
1430  NEXT P
1440  REM ********** PRINT DATA ANALYSIS *******************
1450  IF G(I)<=T+.2 THEN 1470
1460  PRINT "MASS=";J;"CUMULATIVE FIT =";G(I)
1470  LET T=G(I)
1480  NEXT J
1490  LET T=G(I)
1500  LET G(I)=G(I)*R
1510  PRINT "SPECTRUM #=";I;"CUMULATIVE FIT=";T;"WEIGHTED FIT";
          "=";G(I)
1520  LET T=-1
1530  NEXT I
1540  END
```

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